FINAL REPORT

Perchlorate Remediation Using New Nanoscale Polymer Technology

SERDP Project ER-1599

NOVEMBER 2009

Anja Mueller
Central Michigan University/Dendritic Nanotechnologies, Inc./CMU-RC

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
NOV 2009		**00-00-2009 to 00-00-2009**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Perchlorate Remediation Using New Nanoscale Polymer Technology	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Central Michigan University,1200 S. Franklin St,Mount Pleasant,MI,48859**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Principal Ideas Perchlorate has shown carcinogenic, neurodevelopmental, reproductive, and immunotoxic effects. Perchlorate contamination of groundwater is a serious problem at Department of Defense (DOD) facilities nationwide. Ion exchange resins have been used for perchlorate remediation of water; however, the selectivity of these resins is often poor. This leads to low capacity before regeneration of the resin and high salt concentrations needed for regeneration. A scalable dendritic polymer-based filtration material with high capacity for the selective extraction of perchlorate ions from ground water was developed. The resin has binding sites throughout the volume of the resin particle not only on the surface, resulting in large capacity. Summary of Process/Technology Branched dendrigrafts with perchlorate binding sites were synthesized. Crosslinking was performed with poly (ethylene glycol) (PEG) to strengthen the resin and reduce biofouling. Synthesis and purification was optimized for scale-up and cost reduction. Total capacities of the resins were characterized, and initial operating parameters were obtained. This technology is expected to be implemented as a fixed bed system with NaCl regeneration. Brine containing perchlorate and other ions such as nitrate and sulfate from the regeneration process could be disposed of in liquid form, if not inhibited by regulation, or in evaporated form as salt. Results Both uncrosslinked, water-soluble dendrigrafts and poly(ethylene glycol)-crosslinked, water insoluble dendrigrafts were investigated. Resins were tested with high perchlorate concentrations to ensure that even in the presence of high nitrate and sulfate concentrations the capacity for perchlorate is still high. With large capacities the resin is expected to be effective for the removal of low or high concentrations of perchlorate. The highest perchlorate capacities up to 325 meq/g, were found in the soluble dendrigrafts. These dendrigrafts are regenerable with lower concentration brine. Initial toxicity and biofouling data are reported as well. Conclusions Dendrigrafts are an effective way to produce a high perchlorate capacity, regenerable resins with selectivity towards perchlorate. The next step is to determine optimized operational data in a pilot test setting.

15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**87**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

Table of Contents | Page Number

List of Tables

List of Figures

List of Acronyms

amu	Atomic mass unit
BET	Brunauer, Emmett, Teller
Da	Dalton
DI water	Deionized water
DIUF water	Deionized and ultra-filtrated water
DMF	Dimethyl formamide
DoD	Department of Defense
DP	Degree of polymerization
ETSCP	Environmental Security Technology Certification Program
ESI	Electron spin ionization
IR	Infrared
MALDI-TOF	Matrix assisted laser desorption ionization time-of-flight
MeOH	Methanol
MHC	Moisture holding capacity
Mn	Number average molecular weight
MS	Mass spectroscopy
Mw	Weight average molecular weight
MWCO	Molecular weight cut-off
NF	Nanofiltration
NMR	Nuclear magnetic resonance
NOM	Natural organic matter
PBS	Phosphate buffer saline
PEG	Poly (ethylene glycol)
PEI	Polyethyleneimine
PEOX	Polyoxazoline
PVC	Poly (vinylbenzyl chloride)
RO	Reverse osmosis
SEC	Size exclusion chromatography
SERDP	Strategic Environmental Research and Development Program
TLC	Thin layer chromatography
TBAC	Tributyl ammonium chloride
TEAC	Triethyl ammonium chloride
TMAC	Trimethyl ammonium chloride
UF	Ultrafiltration

Keywords:

Perchlorate remediation, dendrigraft, biofouling, total capacity

Acknowledgements:

Help from all DNT employees are highly appreciated.

Abstract

Principal Ideas

Perchlorate has shown carcinogenic, neurodevelopmental, reproductive, and immunotoxic effects. Perchlorate contamination of groundwater is a serious problem at Department of Defense (DOD) facilities nationwide. Ion exchange resins have been used for perchlorate remediation of water; however, the selectivity of these resins is often poor. This leads to low capacity before regeneration of the resin and high salt concentrations needed for regeneration.

A scalable dendritic polymer-based filtration material with high capacity for the selective extraction of perchlorate ions from ground water was developed. The resin has binding sites throughout the volume of the resin particle not only on the surface, resulting in large capacity.

Summary of Process/Technology

Branched dendrigrafts with perchlorate binding sites were synthesized. Crosslinking was performed with poly (ethylene glycol) (PEG) to strengthen the resin and reduce biofouling. Synthesis and purification was optimized for scale-up and cost reduction. Total capacities of the resins were characterized, and initial operating parameters were obtained.

This technology is expected to be implemented as a fixed bed system with NaCl regeneration. Brine containing perchlorate and other ions such as nitrate and sulfate from the regeneration process could be disposed of in liquid form, if not inhibited by regulation, or in evaporated form as salt.

Results

Both uncrosslinked, water-soluble dendrigrafts and poly(ethylene glycol)-crosslinked, water insoluble dendrigrafts were investigated. Resins were tested with high perchlorate concentrations to ensure that even in the presence of high nitrate and sulfate concentrations the capacity for perchlorate is still high. With large capacities the resin is expected to be effective for the removal of low or high concentrations of perchlorate. The highest perchlorate capacities, up to 325 meq/g, were found in the soluble dendrigrafts. These dendrigrafts are regenerable with lower concentration brine. Initial toxicity and biofouling data are reported as well.

Conclusions

Dendrigrafts are an effective way to produce a high perchlorate capacity, regenerable resins with selectivity towards perchlorate. The next step is to determine optimized operational data in a pilot test setting.

Objective

Perchlorate is a persistent environmental contaminant with a variety of health effects[1]. Perchlorate is used as an oxidizer in rocket fuel, fireworks, batteries, and automobile airbags but also occurs in nitrate deposits that are used in fertilizers[2]. Unregulated past releases have contaminated ground water mainly in the western United States. Perchlorate contamination is difficult to treat because it is highly water soluble and does not adsorb well to mineral surfaces[2]. Drinking water standards are still being discussed; they might be changed to levels as low as 4 ppb[2]. Therefore, effective ex-situ clean-up of perchlorate is important. Additionally, DOD facility clean-up might be required as well.

The objectives of this research were to develop a scalable dendritic polymer-based filtration material with high capacity and superior flow rates for the selective extraction of perchlorate ions from ground water. This work was based on the hypothesis that with binding sites throughout the volume of the resin a large capacity would result. This was combined with crosslinking the structure with the antifouling poly(ethylene glycol) (PEG) polymers which would also strengthen the structure towards operating pressures.

The specific technical objectives of this proposal were:

1. Synthesize Priostar® dendrigraft polymers
   Several synthetic routes were evaluated to identify an easily implementable, general, and high yield synthesis towards dendrigrafts.
2. Synthesize, crosslink, and characterize perchlorate-optimized Priostar® dendrigraft polymers
   Several synthetic routes were evaluated to attach a large amount of specific binding sites to the dendrigrafts and to crosslink the substituted dendrigrafts. The chemical structure, amount of substitution, and amount of crosslinking of all dendrigrafts was determined.
3. Scale-up synthesis and purification
   Methods to simplify synthesis and purification of dendrigrafts were identified. Cost analysis determined that these methods considerably reduced preparation costs of dendrigrafts as well.
4. Materials' stability and physicochemical properties
   Besides the chemical structure and the molecular weight of the dendrigrafts, porosity, grain size, biological stability, and biological toxicity were characterized.
5. Measure total capacity
   Total capacity of several dendrigraft backbones, soluble dendrigrafts with several perchlorate binding sites, and crosslinked, insoluble dendrigrafts with several perchlorate binding sites was determined for perchlorate, also for nitrate and sulfate ions.
6. Determine adsorption isotherm and kinetic measurements
   Adsorption isotherms were measured for two dendrigraft backbones.
7. Determine operational properties
   Initial experiments for perchlorate binding under pressure and brine regeneration have been performed.
8. Characterize biofouling

Short-term biofouling measurements with the chosen dendrigraft and bovine serum albumin have been performed.

Background

Perchlorate is a persistent environmental contaminant with a variety of health effects[3] that is in the process of changing regulation. Unregulated past releases, mostly from plants preparing rocket fuel, have contaminated ground water mainly in the western United States; additional natural and industrial sources are being discovered as well. Drinking water standards are still being discussed; they might be changed to levels as low as 4 ppb[4]. Since the rocket fuel sites are operated by the DOD, perchlorate removal from these sites might be required as well
The perchlorate ion is highly soluble in water and highly stable; perchlorate ions have only a very low affinity to sand or resins used in water treatment and thus are difficult to remove from water. Ion exchange resins and microbiological degradation[5, 6] have been used for remediation[4]; here only ion exchange methods are being discussed.

Commercially available ion exchange resins such as Purolite® and Amberlite® resins have been tested for perchlorate sorption[7]. It was determined that a large-sized cation on the resin improved selectivity for perchlorate binding when combined with a less polar binding site. When strong-binding polystyrene resins were used it was not possible to regenerate them with brine solution, the most common regeneration method for ion exchange resins. Tetrachloroferrate was then found to be successful in regenerating these resins[8]. Acrylic type resins, on the other hand, have a higher perchlorate capacity as well as regeneration efficiency[9]. These resins have low perchlorate selectivity, they prefer sulfate over perchlorate and nitrate[10]. Therefore, a new resin based on additional Lewis acid/base forces was developed[11]. For this resin sorption kinetics improved and regeneration was pH dependent. This resin, though, include bound copper ions which were found to leach out of the resin under specific conditions.
Another approach to remove perchlorate from water was to use a dendritic anion host[12]. It is suggested that dendrimers improve the selectivity of perchlorate uptake in the presence of sulfate. It is also hypothesized that the capacity of these resins is larger, thus improving regeneration as well. Large dendrimers as used in that study, though, are still rather expensive.
Selectivity and capacity of ion exchange resins is dependent on the type of resin but even more dependent on the actual binding site[13]. Stronger binding resulted in larger capacity but difficulty to regenerate the resin. The strength of binding was caused by the combination of hydrophobicity of the resin (which resulted in stronger polar forces) and steric hindrance of the binding site[10, 13]. Selectivity towards large anions such as perchlorate was mostly dependent on sterically less hindered quaternary ammonium binding sites[14, 15].

Another difficulty of ion exchange resins is to prevent bio-fouling[16]. Bio-fouling often starts with proteinaceous materials. In medical technology protein binding is commonly prevented by "stealth coatings", generally a coating of poly(ethylene glycol) (PEG) polymers on the surface of the material[17, 18].

In his research regenerable resins with large capacity for perchlorate and low bio-fouling are being made from dendrigraft polymers. Large capacity will be ensured by having accessible binding sites in the inside of the resin particles as well (Fig. 1). This is due to the chemical structure of the dendrigraft polymers that has binding sites along all of the branches of the material. Regeneration will be ensured by choosing weak binding sites. Bio-fouling will be

reduced by including PEG crosslinkers or PEG surface chains in the structure of the branched polymer. This resin modification is also expected to strengthen the physical stability of the dendritic polymers under water pressure.

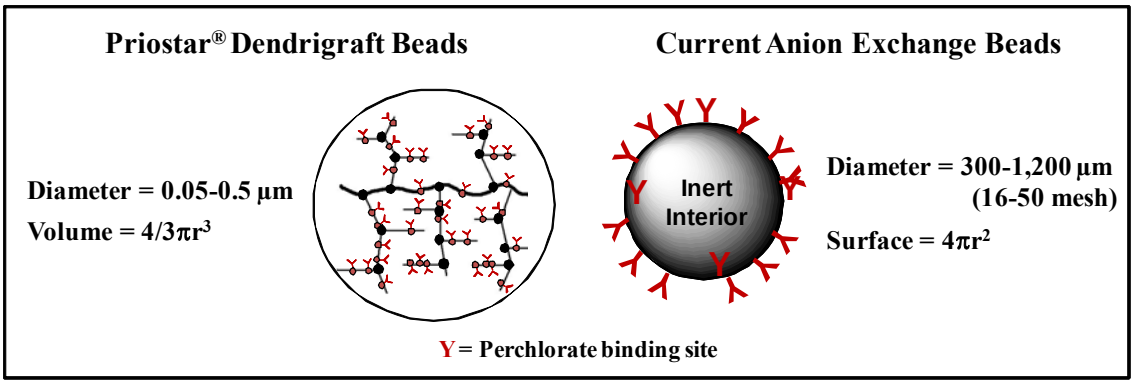


Figure 1. Comparison of active surface area of anion exchange beads with dendrigrafts.

Materials and Methods

1. Synthesis of Priostar® Dendrigraft Polymers

Strategy 1: Experimental Protocols for Dendrigraft Polymers Based on Linear PEI Cores

Preparation of Poly(ethyleneimine) DP=100 from Methyl Tosylate-Initiated Poly(ethyloxazoline) DP= 50, Terminated with Morpholine:

To a 500 mL round bottom flask containing a stir bar and fitted with a Dean – Stark trap and a condenser was added polyethyloxazoline DP = 50 (14 g, 141 mmole PEOX repeat unit) and 100 mL sulfuric acid premixed with 100 mL deionized water. This mixture was refluxed to remove water and propionic acid azeotrope while replacing an equal volume of water. This process was repeated until the water layer in the distillate was not acidic by pH paper. This mixture was cooled to room temperature and 5N NaOH was added to a pH 14. This mixture was briefly boiled and allowed to cool. The product rises to the top of the mixture and solidifies. This solid was removed and added to deionized water. The water was brought to a boil to dissolve the product and the mixture was allowed to cool to room temperature to precipitate the product. This mixture was ultracentrifuged at 4000 rpm for 8 minutes. The clear water layer was decanted off to leave a solid – water mixture that was mixed with toluene and azeotraped to dryness using a Dean – Stark trap. The toluene was volatilized with a rotary evaporator and high vacuum at 85 ºC to give 5.8 g (96 % yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.83 (t), 3.42 (m), 3.16 (t), 2.67 (m), 2.34 (m), 1.08 (m).

MALDI – TOF MS: $C_{13}H_{20}O_8$; Calc. 288.12; found 288 amu.

Preparation of Methyl Tosylate-Initiated Poly(ethyloxazoline) DP= 200:

Methyl p-toluene sulfonate (0.030 g, 0.161 mmol) was added to a 100 ml round-bottom flask, fitted with a magnetic bar, a Dean-Stark trap with a condenser and a nitrogen isolating apparatus, followed by 40 ml of toluene and 15 ml of DMF. The reaction vessel was heated to reflux for a half hour, and the water and the mixture of water and toluene were drained (totally about 10 ml) from Dean-Stark apparatus. The reaction vessel was cooled to 90 ^{o}C, and then 2-ethyl-2-oxazoline (3.194 g, 32.22 mmol) was added. The temperature of the reaction vessel was raised to 110 ^{o}C, and reaction was kept at this temperature for 22 hours. The reaction crude was cooled to 90 ^{o}C, and then morpholine (0.03 g, 0.34 mmol) was added. The temperature of the reaction vessel was raised to 110 ^{o}C again, and reaction was kept at this temperature for 18 hours. Final product was gotten after removal of reaction solvent and non-reacted materials under reduced pressure.

Preparation of Propargyl Tosylate-Initiated Poly(ethyloxazoline) DP= 200:

Propargyl tosylate (0.226 g, 1.142 mmol) was added to a 250 ml round-bottomed flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 150 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 90 ^{o}C, and then 2-ethyl-2-oxazoline (11.323 g, 114.2 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was allowed to keep at this temperature for 30 hours. The reaction crude was cooled to 90 ^{o}C, and then morpholine (0.217 g, 2.5 mmol) was added. The temperature of the reaction vessel was raised to 110 ^{o}C again, and reaction was kept at this temperature for 18 hours. Final product was gotten after removal of reaction solvent and non-reacted materials under reduced pressure.

Grafting Poly(ethyloxazoline) DP= 30, 50% of NH on Poly(ethyleneimine) DP= 50:

Methyl tosylate (1.08g, 5.8 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 75 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed cooled to 100 ^{o}C, and then 2-ethyl-2-oxazoline (17.2 g, 173 mmol) was added to the reaction vessel. The reaction was gently refluxed (~110 ^{o}C) for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP =50 (250 mg, 5.8 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 30 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. To this mixture was added 50 mL methanol and the resulting mixture was refluxed for 2 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 19.8 g crude material. This material was dissolved in 500 mL deionized water to give ~ 4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 30 K regenerated cellulose membranes to give 5 recirculations of permeate (2500 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give g (26 % yield) G = 1 dendrigraft with a molecular weight of ~ 50,000 by size exclusion chromatography or 13 of 50 PEI core NH groups grafted with PEOX DP = 30.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.83 (t), 3.42 (m), 3.16 (t), 2.67 (m), 2.34 (m), 1.08 (m).
^{13}C NMR (75 MHz, ppm, $CDCl_3$) δ: 174.34, 66.71, 65.94, 63.75, 57.04, 53.06, 52.07, 50.49, 43.53, 34.88, 29.44, 25.87, 9.72, 9.36, 9.25.
MALDI – TOF MS: $C_{13}H_{20}O_8$; Calc. 288.12; found 288 amu.

Grafting Poly(ethyloxazoline) DP= 15, 50% of NH on Poly(ethyleneimine) DP= 50:
Methyl tosylate (0.539 mg, 2.9 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 75 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (4.3 g, 43.5 mmol) was added to the reaction vessel. The reaction vessel was gently refluxed (~ 110 ^{o}C), and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP =50 (250 mg, 5.8 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 15 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 5.6 g crude material. This material was dissolved in 150 mL deionized water to give ~ 4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 10 K regenerated cellulose membranes to give 5 recirculations of permeate (800 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give g (26 % yield) G = 1 dendrigraft with a molecular weight of ~ 50,000 by size exclusion chromatography or 13 of 50 PEI core NH groups grafted with PEOX DP = 30.
^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.83 (t), 3.42 (m), 3.16 (t), 2.67 (m), 2.34 (m), 1.08 (m).
^{13}C NMR (75 MHz, ppm, $CDCl_3$) δ: 174.34, 66.71, 65.94, 63.75, 57.04, 53.06, 52.07, 50.49, 43.53, 34.88, 29.44, 25.87, 9.72, 9.36, 9.25.

Grafting Polyethyloxazoline DP = 50, 20 % of NH on Polyethyleneimine DP = 50:
Methyl tosylate (1.08 g, 5.8 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 125 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (29 g, 292.6 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP =50 (1.2 g, 28 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 50 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 35.4 g crude material.

Grafting Poly(ethyloxazoline) DP= 30, 100% of NH on Poly(ethyleneimine) DP= 50:
Methyl tosylate (1.06g, 5.67 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 120 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic

distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (16.8 g, 170 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP = 50 (255 mg, 5.9 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 50 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 18 g crude material. This material was dissolved in 200 mL deionized water to give ~ 4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 30 K regenerated cellulose membranes to give 5 recirculations of permeate (1000 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give 2 g (26 % yield) G = 1 dendrigraft with a molecular weight of ~ 70,000 by size exclusion chromatography or 22 of 50 PEI core NH groups grafted with PEOX DP = 30.

Grafting Polyethyloxazoline DP = 15, 100% of NH on Polyethyleneimine DP = 50:.

Methyl tosylate (1.04 g, 5.60 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 120 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (8.3 g, 84 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP = 50 (252 mg, 5.9 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 15 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 9 g crude material. This material was dissolved in 200 mL deionized water to give ~ 4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 10 K regenerated cellulose membranes to give 5 recirculations of permeate (1000 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give 2g (26 % yield) G = 1 dendrigraft with a molecular weight of ~ 28,000 by size exclusion chromatography or 18 of 50 PEI core NH groups grafted with PEOX DP = 15.

Grafting Poly(ethyloxazoline) DP= 30, 20% of NH on Poly(ethyleneimine) DP= 50:

Methyl tosylate (1.06 g, 5.67 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 120 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (16.8 g, 170 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP = 50 (255 mg, 5.9 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 50 mixture cooled at ~ 100 ^{0}C, and reaction was

gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 18 g crude material. This material was dissolved in 200 mL deionized water to give ~ 4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 30 K regenerated cellulose membranes to give 5 recirculations of permeate (1000 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give 2 g (26 % yield) G = 1 dendrigraft with a molecular weight of ~ 70,000 by size exclusion chromatography or 22 of 50 PEI core NH groups grafted with PEOX DP = 30.

Grafting Poly(ethyloxazoline) DP = 15, 20% of NH on Poly(ethyleneimine) DP= 50:
Methyl tosylate (674mg, 3.62 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 125 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (5.4 g, 54.5 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP = 50 (814 mg, 18.9 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 15 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours.

Grafting Poly(ethyloxazoline) DP = 15, 50% of NH on Poly(ethyleneimine) DP= 50:
Methyl tosylate (526 mg, 2.82 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (4.2 g, 42.3 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added polyethyleneimine DP = 50 (254 mg, 5.9 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 15 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours.

Grafting Poly(ethyloxazoline) DP= 50, 20% of NH on Poly(ethyleneimine) DP= 100:
Methyl tosylate (0.5 g, 2.69 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (13.3 g, 134 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added poly(ethyleneimine) DP= 100 (600 mg, 13.9 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP= 50 mixture cooled at ~100 ^{0}C, and reaction was

gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 14 g crude material. This material was dissolved in 450 mL deionized water to give ~4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 30 KDa regenerated cellulose membranes to give 5 recirculations of permeate (2500 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give 2 g (26 % yield) G= 1 dendrigraft with a molecular weight of ~ 124,000 by size exclusion chromatography or 22 of 50 PEI core NH groups grafted with PEOX DP= 30.

Grafting Poly(ethyloxazoline) DP= 30, 50 % of NH on Poly(ethyleneimine) DP= 100:

Methyl tosylate (1.08 g, 5.81 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (17.2 g, 173.5 mmol) was added to the reaction vessel. The reaction vessel was heated to 110^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added poly(ethyleneimine) DP= 100 (500 mg, 11.6 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP = 100 mixture cooled at ~ 100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. The volatiles were removed by a rotary evaporator and high vacuum at 85 ^{0}C to give 14 g crude material. This material was dissolved in 200mL deionized water to give ~4% solution and ultrafiltered on a tangential flow ultrafiltration device containing 30 K-regenerated cellulose membranes to give 5 recirculations of permeate (1000 mL). The retentate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give 2.96 g (26 % yield) G= 1 dendrigraft with a molecular weight of ~ 112,000 by size exclusion chromatography or 36 of 100 PEI core NH groups grafted with PEOX DP= 30. The remaining material (13 g) was ultrafiltered in the same manner to give 4 g product for a total of 6.96 g product produced from 500 mg PEI core.

Grafting Poly(ethyloxazoline) DP= 30 on Poly(ethyleneimine) DP= 100:

Methyl tosylate (1.08 g, 5.81 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (17.2 g, 173.5 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 24 hours. In a separate 50 mL round bottom flask was added poly(ethyleneimine) DP=100 (500 mg, 11.6 mmol NH) and 40 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 15 minutes. This resulting hot solution was poured into the PEOX DP= 100 mixture cooled at ~100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. Reaction vessel was cooled down to room temperature, and then solvent was removed under reduce pressure to yield product (deep brown viscous liquid).

Partial Hydrolysis of Dendrigraft G= 1, PEI DP= 100 core, PEOX DP= 30 Branch using 0.5 Equivalent of Sulfuric Acid per PEOX Repeat Unit:

To a 100 mL round bottom flask containing a stir bar was added G=1 dendrigraft polymer, PEI DP= 100 core, PEOX DP= 30 branch (3.8 g, 38.3 mmol PEOX repeat unit) and 30 mL deionized water. To this homogeneous solution was added drop wise sulfuric acid (2.35 g, 24 mmol). This mixture was heated at 100 ^{0}C in an oil bath attached to a reflux condenser under N_2 for 6 hours. This mixture was cooled to room temperature and adjusted to a pH= 11.5 using NaOH solution and a pH meter. This mixture was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give a white solid. This mixture was slurried with 100 mL MeOH and gravity filtered with filter paper. The filtrate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give a white solid. This mixture was slurried with 100 mL methylene chloride and gravity filtered. The volatiles were removed from the filtrate by rotary evaporator followed by high vacuum at 95 ^{0}C to give 2.68 g as clear viscous solid. A ^{1}H NMR spectrum analysis indicated 45 % PEI (δ 2.34) and 55 % PEOX (δ 3.34).

Partial Hydrolysis of Dendrigraft G= 1, PEI DP= 100 core, PEOX DP= 30 Branch using 1.0 Equivalent of Sulfuric Acid per PEOX Repeat Unit:

To a 100 mL round bottom flask containing a stir bar was added G=1 dendrigraft polymer, PEI DP = 100 core, PEOX DP = 30 branch (4.0 g, 40.4 mmol PEOX repeat unit) and 30 mL deionized water. To this homogeneous solution was added drop wise sulfuric acid (3.9g, 39.8 mmol). This mixture was heated at 100 ^{0}C in an oil bath attached to a reflux condenser under N_2 for 6 hours. This mixture was cooled to room temperature and adjusted to a pH = 11.5 using NaOH solution and a pH meter. This mixture was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give a white solid. This mixture was slurried with 100 mL MeOH and gravity filtered with filter paper. The filtrate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give a white solid. This mixture was slurried with 100 mL methylene chloride and gravity filtered. The volatiles were removed from the filtrate by rotary evaporator followed by high vacuum at 95^{0}C to give 2.28 g as clear viscous solid. A 1H NMR spectrum analysis indicated 58 % PEI (δ 2.34) and 42 % PEOX (δ 3.34).

Partial Hydrolysis of Dendrigraft G= 1, PEI DP= 100 core, PEOX DP= 30 Branch using 2.0 Equivalent Sulfuric Acid per PEOX Repeat Unit:

To a 100 mL round bottom flask containing a stir bar was added G=1 dendrigraft polymer, PEI DP= 100 core, PEOX DP= 30 branch (4.0 g, 40.4 mmol PEOX repeat unit) and 30 mL deionized water. To this homogeneous solution was added drop wise sulfuric acid (7.9 g, 80.6 mmol). This mixture was heated at 100 ^{0}C in an oil bath attached to a reflux condenser under N_2 for 6 hours. This mixture was cooled to room temperature and adjusted to a pH= 11.5 using NaOH solution and a pH meter. This mixture was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give a white solid. This mixture was slurried with 100 mL MeOH and gravity filtered with filter paper. The filtrate was evacuated of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give a white solid. This mixture was slurried with 100 mL methylene chloride and gravity filtered. The volatiles were removed from the filtrate by rotary evaporator followed by high vacuum at 95 ^{0}C to give 950 mg as clear viscous solid. A ^{1}H NMR spectrum analysis indicated 88 % PEI (δ 2.34) and 12 % PEOX (δ 3.34).

Preparation of Dendrigraft Polymer G1(100-30):

Methyl tosylate (15.43 g, 83.0 mmol) was added in a 2-L round bottom flask fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubbler, followed by 1100 mL of toluene. The reaction vessel was heated to reflux for 30 min to remove water by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and 2-ethyl-2-oxazoline (242.0 g, 2.24 mol) was added to the reaction vessel. The vessel was heated under gentle reflux (~110 ^{o}C) for 24 hours. In a separate 250-mL round bottom flask was added polyethyleneimine (PEI) DP= 100 (7.2 g, 167 mmol NH) and 150 mL of toluene. The mixture was dissolved and dried by refluxing the toluene through a Dean-Stark trap for 30 minutes. This hot solution was poured into the PEOX DP= 30 mixture, cooled to ~100 ^{o}C, and kept at gentle reflux under N_2 gas for 24 hours. The reaction vessel was cooled to room temperature, and volatiles were removed by rotary evaporation followed by high vacuum distillation at 85 ^{o}C to give 216.0 g of crude product. This product was split into two equal portions, each portion dissolved in 1500 mL of deionized water, and purified by ultrafiltration on a tangential flow ultrafiltration device at a pressure of 25 psi using a 30 kDa cut-off regenerated cellulose membrane. After 5 recirculations, the retentate was stripped of volatiles on a rotary evaporator followed by high vacuum distillation at 85 ^{o}C to give 48.8 g of G1 dendrigraft product. All unreacted PEOX DP= 30 had been removed as verified by SEC. The second half of the crude material gave 52.0 g of G1 product under the same conditions, yielding a total of 101.0 g of G1(100-30).

To a 1 L round bottom flask containing a stir bar and fitted with a Dean-Stark trap and a condenser was added dendrigraft polymer G1(100-30) (50.0 g, 504.0 mmol PEOX repeat unit) and 53.0 mL of sulfuric acid, premixed with 530 mL of deionized water. This mixture was refluxed to remove water and propionic acid as an azeotrope mixture, while replacing the volume with an equal amount of water. This process was repeated to remove 500 mL of distillate. The resulting mixture was heated to reflux for 26 hours. This mixture was cooled to room temperature and the pH adjusted to 14 by addition of 5N NaOH. The mixture was briefly boiled and again allowed to cool to room temperature. The product rose to the top of the mixture and solidified upon cooling. The solid was removed and deionized water was added. The water was boiled to dissolve the product and the mixture was allowed to cool to room temperature to precipitate the purified product. Dendrigraft polymer G1(100-30) was isolated by ultracentrifugation at 4000 rpm for 8 minutes. The clear water layer was decanted off and replaced by fresh water. After a second ultracentrifugation and separation, the remaining water-dendrigraft mix was diluted with toluene and all water removed as toluene azeotrope using a Dean-Stark trap. The toluene mixture was filtered through a Whatman No. 1 filter paper and dried by rotary evaporation and high vacuum drying at 85 ^{o}C to give 16.0 g (96% theoretical yield) of G1(100-30). The grafting density of PEI 30 along the PEI 100 core was ~37%.

Preparation of Dendrigraft Polymer G1(200-60) – 1.0 Equiv.

Following the above protocol, core PEI 200 was reacted with branching agent PEOX 60 (1.0 equiv. per NH group of PEI 200) to give dendrigraft polymer G1(200-60). Materials used for the reaction were methyl tosylate (3.165 g, 17.0 mmol) in 600 mL of toluene and 2-ethyl-2-oxazoline (101.27 g, 1.02 mol); and PEI 200 (730.0 mg, 17.0 mmol NH) in 40 mL of toluene.

Dendrigraft polymer G1(200-60) was isolated after ultrafiltration in a yield of 23.45 g (22% grafting yield). The molecular weight determined by SEC of the dendrigraft product was ~280,000 Da, with a grafting yield of 22% or 40 NH sites of 200 available NH sites had been grafted.

Preparation of Dendrigraft Polymer G1(700-200) – 1.0 Equiv.

Following the above protocol, core PEI 700 was reacted with branching agent PEOX 200 (1.0 equiv. per NH group of PEI 200) to give dendrigraft polymer G1(700-200). Materials used for the reaction were methyl tosylate (650.0 mg, 3.5 mmol) in 150 mL of toluene/125 mL anhydrous DMF and 2-ethyl-2-oxazoline (70.0 g, 706.0 mmol); and PEI 700 (150.0 mg, 3.5 mmol NH) in 40 mL of toluene. Dendrigraft polymer G1(700-200) was isolated after ultrafiltration in a yield of 4.3 g (22% grafting yield). The molecular weight determined by SEC of the dendrigraft product was ~150,000 Da, with a grafting yield of 8% or 49 NH sites of 700 available NH sites had been grafted.

Preparation of Dendrigraft Polymer G1(700-200) – 0.5 Equiv.

Following the above protocol, core PEI 700 was reacted with branching agent PEOX 200 (0.5 equiv. per NH group of PEI 200) to give dendrigraft polymer G1(700-200). Materials used for the reaction were methyl tosylate (650.0 mg, 3.5 mmol) in 150 mL of toluene/125 mL anhydrous DMF and 2-ethyl-2-oxazoline (70.0 g, 706.0 mmol); and PEI 700 (300.0 mg, 7.0 mmol NH) added as a solid to the PEOX 200 solution. Dendrigraft polymer G1(700-200) was isolated after ultrafiltration in a yield of 5.5 g. The molecular weight determined by SEC of the dendrigraft product was essentially the same as in the reaction using 1.0 equivalent PEOX 200 (~150,000 Da), with a grafting yield of 8% or 49 NH sites of 700 available NH sites had been grafted.

Preparation of Dendrigraft Polymer G1(700-100) – 0.5 Equiv.

Following the above protocol, core PEI 700 was reacted with branching agent PEOX 100 (0.5 equiv. per NH group of PEI 100) to give dendrigraft polymer G1(700-100). Materials used for the reaction were methyl tosylate (1.3 g, 7.0 mmol) in 200 mL of toluene and 2-ethyl-2-oxazoline (70.0 g, 706.0 mmol); and PEI 700 (600.0 mg, 14.0 mmol NH) in 40 mL of toluene. Dendrigraft polymer G1(700-100) was isolated after ultrafiltration in a yield of 5.5 g (17% grafting yield).

Preparation of Dendrigraft Polymer G2(100-30-15)

Methyl tosylate (20.2 g, 108.6 mmol) was added in a 500-mL round bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 200 mL of toluene. The reaction vessel was heated to reflux for a half hour, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100^{o}C, and 2-ethyl-2-oxazoline (161.6 g, 1.63 mol) was added to the reaction vessel. The vessel was heated under gentle reflux (~110 ^{o}C) for 24 hours. In a separate 100-mL round bottom flask was added dendrigraft polymer G1(100-30**)** **(**4.67 g, 108.6 mmol NH**)** in 60 mL of toluene. The mixture was dissolved and dried by refluxing the toluene through a Dean-Stark trap for 15 minutes. This hot solution was poured into the PEOX DP= 15 mixture, cooled to ~100 ^{o}C, and kept at gentle reflux under N_2 gas for 24 hours. The reaction vessel was cooled to room temperature, and volatiles were removed by rotary

evaporation to give 106 g crude material. The crude material was dissolved in a mixture of methanol and water to give a homogeneous solution. Purification was carried out by ultrafiltration using a tangential ultrafiltration device containing 30 kDa cut-off regenerated cellulose membrane. After eight recirculations, the retentate was stripped of volatiles on a rotary evaporator followed by high vacuum drying to give dendrigraft polymer G2(100-30-15) in a yield of 44.2 g (17% grafting yield).

Preparation of Dendrigraft Polymer G2(200-60-30)

Following the above protocol, dendrigraft polymer G1(200-60) was reacted with branch reagents PEOX 30 to give dendrigraft polymer G2(200-60-30). Materials used for the reaction were methyl tosylate (1.86 g, 10.0 mmol) in 180 mL of toluene and 2-ethyl-2-oxazoline (29.76 g, 300.0 mmol); and dendrigraft polymer G1(200-60) (537.5 mg, 12.5 mmol NH) in 60 mL of toluene. Dendrigraft polymer G2(200-60-30) was isolated after ultrafiltration in a yield of 7.83 g (18% grafting yield).

Strategy 2. Experimental Protocols for Dendrigraft Polymers Based on Microsphere Cores

Preparation of Polybenzyl Chloride Microspheres:

To a 500 mL 3-neck round bottom flask fitted with a reflux condenser attached to a N_2 bubbler, a mechanical stirrer was added a solution prepared from polyvinyl alcohol (500 mg) in 160 mL deionized water by heating to 88 ^{0}C with stirring until the mixture was homogeneous. To this mixture stirred 700 rpm (as determined by a Strobe light) at 78 ^{0}C was added all at once a solution of vinyl benzyl chloride (10 mL, 63.8 mmol), ethylene glycol dimethacrylate (3.0 mL, 15.6 mmol) and azobisisobutyronitrile (240 mg, 1.4 mmol) dissolved in 15 mL heptane. This mixture was stirred for 8 hours under N_2. This mixture was poured into 500 mL water and filtered through fast flow filter paper in a Buchner funnel, washed with 1L water and dried under high vacuum to a constant weight of 12 g.

Conversion of Polybenzyl Chloride Microspheres to Piperazine Surface Microspheres:

To a 250 mL round bottom flask containing a stir bar was added piperazine (4.5 g, 52.3 mmol) and potassium carbonate (7.5 g, 54.3 mmol) and 60 mL anhydrous DMF. This mixture was stirred and heated at 60^{0}C until all the piperazine was dissolved. To this mixture poly(benzyl chloride) microspheres (2.0 g) was added. This mixture was stirred and heated at 60 ^{0}C for 3 days. This mixture was poured into 500 mL deionized water and filtered through Whatman No. 1 filter paper in a large Buchner funnel. This solid was washed with 1 L water followed by 500 mL MeOH. This solid was placed in a flask and evacuated at high vacuum to give a constant weight of 2.3 g.

IR: cm^{-1} (Neat) 2929, 2100, 1731, 1608, 1449, 1342, 1260, 1106.

Conversion of Polybenzyl Piperazine Microspheres to Phenyl Ureamide Surface Microspheres:

To a 10 mL round bottom flask containing a stir bar was added polybenzyl piperazine surface microspheres (100 mg) and phenyl isocyanate (300 mg, 54.3 mmol) and 3 g anhydrous DMF. This mixture was stirred at room temperature overnight. This mixture was poured into 20 ml MeOH and filtered through Whatman No. 1 filter paper in a small Buchner funnel. This solid

was washed with 100 mL MeOH and air dried to solid was placed in a flask and evacuated at high vacuum to give a constant weight of 2.3 g.
IR: cm^{-1} (Neat) 2929, 2100, 1731, 1608, 1449, 1342, 1260, 1106.

Reaction of Polybenzyl Piperazine Microspheres with Methyl Tosylate-Initiated Poly(oxazoline) DP= 20:
To a 250 mL round bottom flask containing a large stir bar was added methyl tosylate (1.42 g, 7.6 mmol) and 100 mL toluene. This flask was attached to a Dean – Stark trap and a reflux condenser under a N_2 bubbler. This mixture was gently refluxed to remove 20 – 30 mL toluene – water to dry the system over 30 minutes. This mixture was cooled to about 80 ºC and the trap removed replacing with the reflux condenser. Ethyl oxazoline (15 g, 151 mmol) was added to this mixture and the resulting material was gently refluxed at 110 ^{0}C for 5 hours. A TLC (MeOH) of this mixture indicated no more monomer remaining. To this mixture cooled to ~90 ^{0}C was added polybenzyl chloride microspheres with piperazine surface (1.0) as a solid. This mixture was gently refluxed for 18 hours. To this mixture was added 10 mL water and the resulting mixture refluxed for 1 hour. The mixture was cooled and added to 100 mL MeOH and filtered in a large Buchner funnel fitted with fast flow filter paper. The solid was washed with 500 mL water and 500 mL MeOH, air dried for 2 – 3 hours then evacuated at room temperature at high vacuum to a constant weight of 900 mg.
IR: cm^{-1} (Neat) 2929, 2100, 1731, 1654, 1449, 1296, 1157, 1096, 1029.

Reaction of Polybenzyl Piperazine Microspheres with Methyl Tosylate-Initiated Poly(oxazoline) DP= 100:
To a 250 mL round bottom flask containing a large stir bar was added methyl tosylate (300 mg, 1.6 mmol) and 100 mL toluene. This flask was attached to a Dean – Stark trap and a reflux condenser under a N_2 bubbler. This mixture was gently refluxed to remove 20 – 30 mL toluene – water to dry the system over 30 minutes. This mixture was cooled to about 80 ^{0}C and the trap removed replacing with the reflux condenser. Ethyl oxazoline (15 g, 151 mmol) was added to this mixture and the resulting material was gently refluxed at 110 ^{0}C for 18 hours. A TLC (MeOH) of this mixture indicated no more monomer remaining. To this mixture cooled to ~90 ^{0}C was added polybenzyl chloride microspheres with piperazine surface (1.0) as a solid. This mixture was gently refluxed for 18 hours. To this mixture 10 mL water was added and the resulting mixture was refluxed for 1 hour. The mixture was cooled and added to 100 mL MeOH and filtered in a large Buchner funnel fitted with fast flow filter paper. The solid was washed with 500 mL water and 500 mL MeOH, air dried for 2 – 3 hours then evacuated at room temperature at high vacuum to a constant weight of 900 mg.
IR: cm^{-1} (Neat) 2929, 2100, 1731, 1654, 1449, 1296, 1157, 1096, 1029.

Conversion of Polybenzyl Chloride Microspheres to Azide Surface Microspheres:
Polybenzyl chloride microsphere beads (300 mg) were added to a 50 ml round-bottom flask with a N_2 bubbler and a magnetic stir bar, followed by DMF (13.5 ml), deionized water (1.5 ml), and sodium azide (150 mg, 2.3 mmol). Reaction was run for 24 hours at room temperature. The solution was removed by Buchner funnel. The solid beads was washed with a large amount of water (150-200 mL), followed by methanol (70-100 mL), and then dried under reduced pressure.
IR: cm^{-1} (Neat) 2929, 2100, 1731, 1608, 1449, 1342, 1260, 1106.

Preparation of Propargyl Tosylate-Initiated Poly(ethyloxazoline) DP= 10:

Propargyl tosylate (2.0 g, 10 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for a half hour, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 50 ^{o}C, and then 2-ethyl-2-oxazoline (9.91 g, 100 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 18 hours. Morpholine (2.0 g, 22 mmol) was added quickly, and reaction was continued under same condition for another 18 hours. Reaction vessel was cooled down to room temperature, and then solvent was removed under reduce pressure to yield product (deep brown viscous liquid).

^{1}H NMR (300 MHz, ppm, $CDCl_3$) δ: 3.83 (t), 3.42 (m), 3.16 (t), 2.67 (m), 2.34 (m), 1.08 (m).

^{13}C NMR (75 MHz, ppm, $CDCl_3$) δ: 174.34, 66.71, 65.94, 63.75, 57.04, 53.06, 52.07, 50.49, 43.53, 34.88, 29.44, 25.87, 9.72, 9.36, 9.25

MALDI – TOF MS: $C_{13}H_{20}O_8$; Calc. 288.12; found 288 amu.

Preparation of Propargyl Tosylate Initiated Poly(ethyloxazoline) DP= 50:

Propargyl tosylate (0.396 g, 2 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 50 ^{o}C, and then 2-ethyl-2-oxazoline (9.91 g, 100 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 18 hours. Morpholine (0.35 g, 4 mmol) was added quickly, and reaction was continued under same condition for another 18 hours. Reaction vessel was cooled down to room temperature, and then solvent was removed under reduce pressure to yield product (brown solid).

^{1}H NMR (300 MHz, ppm, $CDCl_3$) δ: 3.83 (t), 3.44 (s), 3.16, 2.33 (m), 1.09 (t).

^{13}C NMR (75 MHz, ppm, $CDCl_3$) δ: 174.44, 174.34, 173.85, 77.20, 76.29, 64.45, 50.64, 46.17, 45.74, 45.38, 43.99, 43.59, 25.90, 9.37, 9.28

MALDI – TOF MS: $C_{13}H_{20}O_8$; Calc. 288.12; found 288 amu.

Preparation of Propargyl Tosylate-Initiated Poly(ethyloxazoline) DP= 100:

Propargyl tosylate (0.10 g, 0.50 mmol) was added to a 250 ml round-bottomed flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 50 ^{o}C, and then 2-ethyl-2-oxazoline (4.96 g, 50 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ^{o}C, and reaction was kept at this temperature for 18 hours. Morpholine (0.09 g, 1.0 mmol) was added quickly, and reaction was continued under same condition for another 18 hours. Reaction vessel was cooled down to room temperature, and then solvent was removed under reduce pressure to yield product (brown solid).

^{1}H NMR (300 MHz, ppm, $CDCl_3$) δ: 3.82 (t), 3.43 (s), 3.14, 2.33 (m), 1.09 (s).

^{13}C NMR (75 MHz, ppm, $CDCl_3$) δ: 174.41, 174.31, 173.81, 77.20, 76.30, 56.96, 53.21, 46.92, 46.14, 45.73, 45.41, 43.96, 43.75, 43.40, 25.89, 9.34.
MALDI – TOF MS: $C_{13}H_{20}O_8$; Calc. 288.12; found 288 amu.

Reaction of Polybenzyl Azide Microspheres with Propargyl Tosylate-Initiated Poly(oxazoline) DP= 10:

PEOX (DP= 10; 0.13 g, initiated by propargyl tosylate (2-propyn-1-ol-4-methylbenezene sulfonate) and terminated by morpholine) was dissolved into 20 mL of mixture of DMF and deionized water (1:1, v/v), followed by azide-terminated micro sphere beads (0.17 g), aqueous copper(II) sulfate solution (52 mg of copper(II) sulfate pentahydrate (0.2 mmol) dissolved in 5 mL of deionized water), and aqueous L-ascorbic acid sodium salt solution (82 mg of L-ascorbic acid sodium salt (0.4 mmol) dissolved in 5 mL of deionized water). The reaction was carried out under temperature of 50 ^oC for 16 hours. The reaction solution was removed by Buchner funnel. The solid beads was washed with a large amount of deionized water (450-500 mL), followed by methanol (150-200 mL), and then dried under reduced pressure.
IR: cm^{-1} (Neat) 2934, 2100, 1731, 1649, 1454, 1347, 1265, 1116.

Reaction of Polybenzyl Azide Surface Microspheres with Propargyl Tosylate-Initiated Poly(oxazoline) DP= 50:

PEOX (DP= 50; 0.43 g, initiated by propargyl tosylate (2-propyn-1-ol-4-methylbenezene sulfonate) and terminated by morpholine) was dissolved into 20 mL of mixture of DMF and deionized water (1:1, v/v), followed by azide-terminated micro sphere beads (0.20 g), aqueous copper(II) sulfate solution (52 mg of copper(II) sulfate pentahydrate (0.2 mmol) dissolved in 5 mL of deionized water), and aqueous L-ascorbic acid sodium salt solution (82 mg of L-ascorbic acid sodium salt (0.4 mmol) dissolved in 5 mL of deionized water). The reaction was carried out under temperature of 50 ^oC for 16 hours. The reaction solution was removed by Buchner funnel. The solid beads was washed with a large amount of deionized water (450-500 mL), followed by methanol (150-200 mL), and then dried under reduced pressure.
IR: cm^{-1} (Neat) 2929, 2100, 1731, 1675, 1654, 1449, 1388, 1270, 1111.

Reaction of Benzyl Azide Surface Microspheres with Propargyl Tosylate-Initiated Poly(oxazoline) DP= 100:

PEOX (DP 100, 0.83 g, initiated by propargyl tosylate (2-propyn-1-ol-4-methylbenezene sulfonate) and terminated by morpholine) was dissolved into 20 mL of mixture of DMF and deionized water (1:1, v/v), followed by azide-terminated micro sphere beads (0.20 g), aqueous copper(II) sulfate solution (52 mg of copper(II) sulfate pentahydrate (0.2 mmol) dissolved in 5 mL of deionized water), and aqueous L-ascorbic acid sodium salt solution (82 mg of L-ascorbic acid sodium salt (0.4 mmol) dissolved in 5 mL of deionized water). The reaction was carried out under temperature of 50 ^oC for 40 hours. The reaction solution was removed by Buchner funnel. The solid beads was washed with a large amount of deionized water (450-500 mL), followed by methanol (150-200 mL), and then dried under reduced pressure.
IR: cm^{-1} (Neat) 2929, 2100, 1731, 1654, 1449, 1296, 1157, 1096, 1029

2. Synthesis, Crosslinking, and Characterization of Perchlorate-Optimized Priostar® Dendrigraft Polymers

Reaction between Poly(ethyleneimine) and Glycidyl Trimethylammonium Chloride (TMAC):

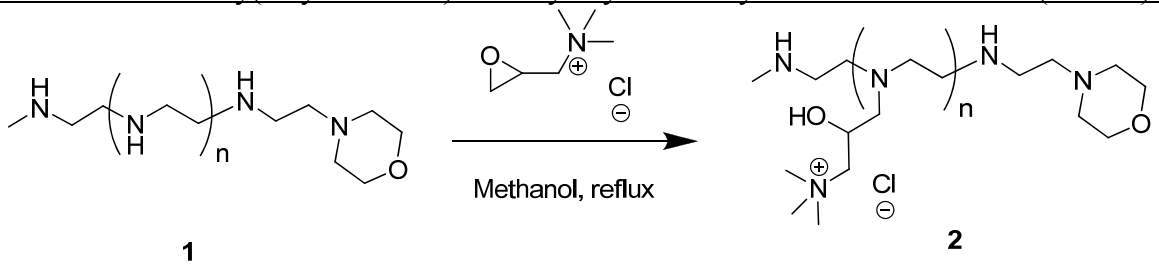


Figure 2. Reaction between poly(ethyleneimine) and glycidyl trimethylammonium chloride (TMAC) to synthesize TMAC-substituted resin.

Linear poly(ethyleneimine) PEI DP= 100 (**1**, 0.4 g, 9.3mmol -NH) was dissolved in 20 mL methanol and glycidyl TMAC (1.6 g, 10.6mmol) was added. The mixture was then refluxed for 12 hours and dried to yield a white sticky mass. The residue was dissolved in water and purified by ultrafiltration (MWCO = 1K). Then retentate was dried to give a thick mass **2** (1.0g). It was characterized by NMR and SEC.
^{1}H NMR (300MHz, D_2O): δ = 2.45 (2H), 2.6 (4H), 3.05 (9H), 3.25 (2H), 4.2 (1H); SEC: MP = 31091; PDI = 1.14.

Reaction between Epichlorohydride and Glycidyl Triethylammonium Chloride (TEAC):
Epichlorohydrin **3** (43.2g) and triethylamine **4** (43.2g) were mixed together and stirred under room temperature for 4 days. The mixture separated into two phases. The lower phase was separated and dried by high vacuum to give a semi-crystalline residue glycidyl TEAC **5** (9.8 g).

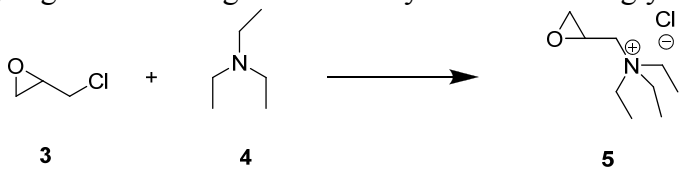
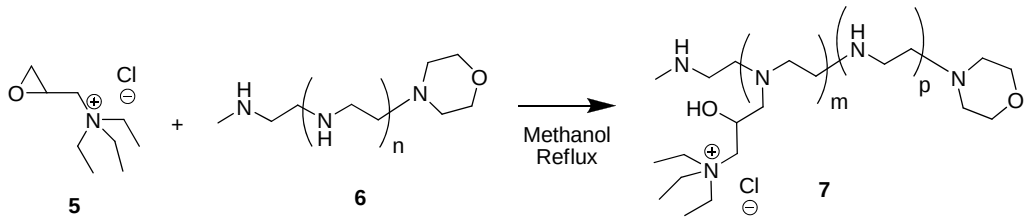


Figure 3. Reaction between epichlorohydrin and glycidyl TEAC to synthesize TEAC binding site with glycidyl reactive site.

Reaction between Poly(Ethylene Imine) and Glycidyl TEAC:
Crude product **5** (0.59g) was dissolved in 10 mL methanol and 0.086 g PEI-DP100 was added. The mixture was refluxed for 12 hours. The solution was then diluted with water and purified by ultrafiltration (MWCO = 10K). The retentate was dried to give a thick mass **7** (0.3 g). It was characterized by NMR and SEC.
^{1}H NMR (300MHz, D_2O): δ = 1.1 (7.35H) 2.3-2.8 (8.9H), 3.0-3.5 (8.6H), 4.0-4.1 (1H); SEC: MP = 10782; PDI = 1.25.



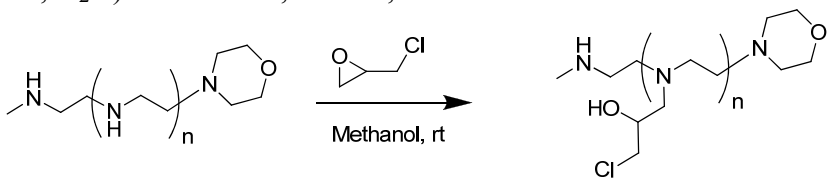
5 6 7

Figure 4. Reaction between PEI and TMAC binding site.

Reaction between Poly(Ethyleneimine) and Epichlorohydrin:

PEI-DP100 (1.01g) was mixed with epichlorohydrin (3.18g) in 20 mL MeOH. The mixture was stirred under room temperature for 16 hours. The mixture was dried to yield a solid mass **8** (4.02 g).

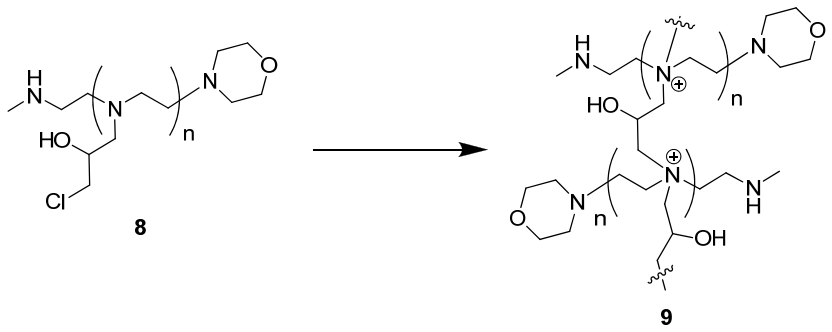
^{1}H NMR (300MHz, D_2O): δ = 2.5-2.8, 3.5-3.7, 3.8-4.0.



6 8

Figure 5. Reaction between PEI and epichlorohydrin to add binding site attachment points to PEI.

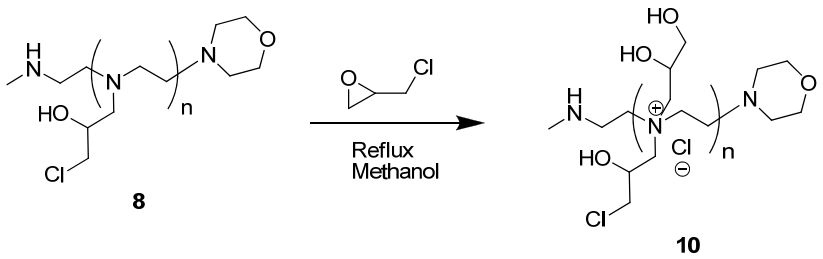
When compound **8** was dried and heated, the material became insoluble in all common solvents, including water, methanol, chloroform, etc. This material, however, absorbs large amount of water and forms a gel. It is possible that self-crosslinking happened to form a hydrophilic network.



8 9

Figure 6. Possible major product of the above reaction.

When compound **8** is heated with more epichlorohydrin in a solvent, such as methanol, it continues to react with epichlorohydrin to form a soluble material. The reaction is possibly through the following pathway:

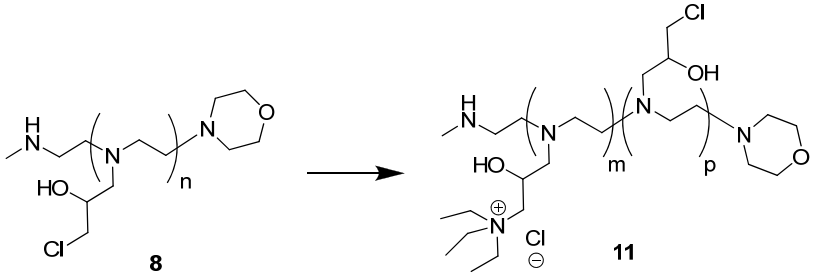


8 10

Figure 7. Possible major product of above reaction.

^{1}H NMR (300MHz, D_2O): δ = 2.4-3.2(b), 3.3-4.4

Alternate Route to Add Quaternary Ammonium Cation:



8 11

Figure 8. Alternate route to add trialkylammonium binding site to PEI.

Due to the low reactivity between epichlorohydrin and trialkylamine for longer alkyl chains, it is relatively difficult to synthesize glycidyltrialkylammonium chloride. We therefore tried another route, i.e., to treat PEI with epichlorohydrin followed by trialkylamine. Compound **8** (0.4g) was boiled with 40 mL methanol and 10 mL triethylamine for 16 hours. The resulted solution was dried and purified by ultrafiltration (MWCO = 10K). The retentate was dried to yield compound **11** as a sticky liquid.
For example for TEAC: ^{1}H NMR (300MHz, D_2O): δ = 1.1, 2.2-4.2 (b).

NMR suggested the installation of new ethyl group by the new peak at 1.1 ppm. The NMR profile is, however, significantly different from the product **7** obtained from the direct reaction between PEI-DP100 and pre-prepared glycidyl triethylammonium chloride. NMR of the latter has more defined peaks and can be easily assigned.

To further elucidate the structure, PEI-DP100 was reacted with the mixture (~1:1) of epichlorohydrin and glycidyl TEAC and the product **12** was compared with compound **11**. The highly similar NMR profiles indicate that the two-step route actually results in a more complex backbone therefore more complex NMR profile. It also has serious amount of quaternary charge installed, although less than the direct reaction between PEI-DP100 and glycidyl TEAC.

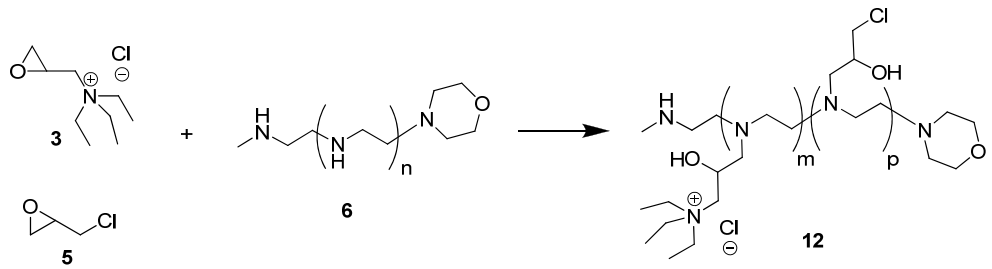


Figure 9. Possible major product of reaction above.

Synthesis of Tributyl Ammonium Chloride (TBAC):

The third binding site of interest, glycidyl TBAC was synthesized by reaction of epichlorohydrin and tributylamine, both of which are commercially available inexpensive starting materials. Epichlorohydrin (22.7 g) and tributylamine (50 mL) were mixed in 200 mL of methanol in a 250-mL round bottom flask. The mixture was heated to reflux in an oil bath (~65^{o}C) for 3 hours and dried under high vacuum to yield the product in 39.4 g (58% of theory) as oil.
^{1}H NMR (300MHz, D_2O): δ = 1.0 (9H, t), 1.4 (6H, m), 1.7 (6H, m), 2.7 (1H, dd), 2.95(1H, dd), 3.1 (1H, dd) 3.4 (9H, m), 3.9 (1H, dd) ppm.

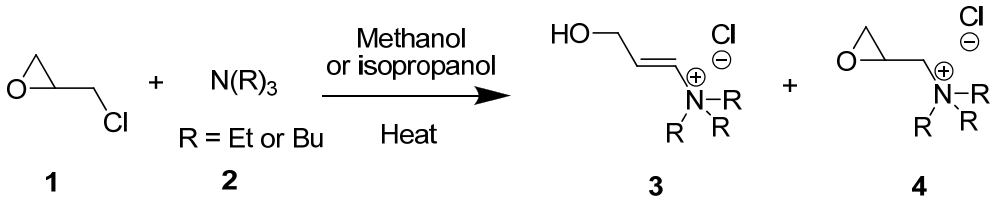


Figure 10. The preparation of glycidyltrialkylammonium chlorides.

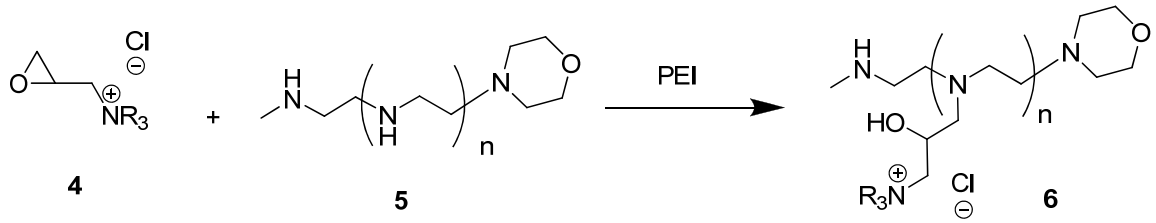


Figure 11. The reaction of glycidyltrialkylammonium chlorides with PEI.

Example for the Reaction between Binding Sites and PEI:

Epichlorohydrin (2.0 g) and tributylamine (4.4 g, 1.1 equiv.) were mixed with 20 mL of methanol, heated to reflux for 2 hours, and dried by rotary evaporation. After drying and cooling, the residue separated into two phases. The upper phase (tributylamine) was discarded, while the lower phase contained the product glycidyl tributylammonium chloride. To this residue, 20 mL of methanol and PEI 50 (0.35 g, 0.9 equiv.) were added. The mixture was heated to reflux for 16 hours and dried by rotary evaporation. The residue was dissolved in 200 mL of water and purified by ultrafiltration (MWCO 1 kDa). The retentate was analyzed by NMR.

^{1}H NMR (300MHz, D_2O)**:** δ = 0.8 (9H, b), 1.2 (6H, b), 1.5 (6H, b), 2.2-2.9 (9H, b), 3.0-3.6(9H, b), 4.0 (1H, b).

Preparation of Crosslinked G1(100-30)-TMAC:
To a 25 mL two neck round bottom flask fitted with a mechanical stirrer and a N_2 bubbler was added dendrigraft G1(100-30) functionalized with 30 % glycidyl TMAC (1.6 g) dissolved in 9 g isopropanol (15 wt % solution). To this mixture was added polyethyleneglycol diglycidyl ether (MW = 252) (240 mg, 15 wt %). This resulting mixture was heated with stirring for 2.5 – 3 hours during which the viscous liquid changed to a solid product.

Preparation of Glycidyl Triethyl Ammonium Chloride (TEAC):
To a 250 mL round bottom flask containing a stir bar was added triethylamine (10 g, 99 mmol), epichlorohydrin (13.74 g, 149 mmol, 1.5 equivalents) and 40 mL methanol. This mixture was stirred and refluxed for 2.5 hours. This mixture was cooled and evacuated of volatiles using a rotary evaporator followed by high vacuum for a maximum of 2 hours give 19.7 g (98 % yield) of the title compound.

Preparation of Glycidyl TBAC:
To a 250 mL 2 neck round bottom flask containing a stir bar and fitted with a dropping funnel and reflux condenser was added epichlorohydrin (20 g, 216 mmol, 2.5 equivalents) and 20 mL methanol. The resulting mixture was heated to 55 ^{0}C. Tributylamine (16 g, 86.5 mmols) and 16 mL MeOH were added to the dropping funnel and slowly added drop wise to the reaction mixture over 30 minutes. This resulting mixture was stirred at 55 ^{0}C for 5 hours. This mixture was cooled to room temperature and evaporated of volatiles using ac rotary evaporator. The resulting residue formed two layers containing the amine in the upper layer and the product in the lower layer. The upper layer was washed out with 3 x 20 mL hexanes. The extracted residue was evacuated at high vacuum to yield 23.2 g (96 % yield) product.

Preparation of G1(100-30)-TMAC:
To a 500 mL round bottom flask with a stir bar was added dendrigraft G = 1 PEI (100, 30) (10.25 g, 238 mmol NH) and glycidyl TMAC (17.99 g, 119 mmol, 50 % per NH) and 180 mL MeOH. This mixture was refluxed for 16 hours. The volatiles were removed by rotary evaporator and the resulting residue dissolved in 400 mL deionized water. This mixture was purified using a tangential flow ultrafiltration device containing a 3 K regenerated cellulose membrane collecting 3.2 L of permeate (8 recirculations). The retentate was stripped of volatiles to give 26.1 g (43 % grafting).

Preparation of G1(100-30)-TEAC:
G1(100-30) PEI (1.0 g, 23.25 mmoles of NH groups) and glycidyl TEAC (1.84 g, 9.5, mmoles, 40 % per NH) were dissolved in 40 mL of methanol in a 100 mL round bottom flask. The reaction vessel was refluxed for 16 hours, and then stripped to remove solvent. The crude product was dissolved in 200 mL water, and purified by ultrafiltration. (1K or 3K, Every circulation was from 150 mL to 50 mL, total needs 9 circulations). After UF, the solution was stripped to yield 2.0 g product. (27 % grafting)

Preparation of G1(100-30)-TBAC:
G1(100-30) PEI (1.0 g, 23.25 mmoles of NH groups) and glycidyl TBAC (2.25 g, 9.29 mmoles) were dissolved in 40 mL of methanol in a 100 mL round bottom flask. The reaction vessel was refluxed for 16 hours, and then stripped to remove solvent. The crude product was dissolved in 200 mL water, and purified by ultrafiltration. (1K or 3K, Every circulation was from 150 mL to 50 mL, total needs 9 circulations). After UF, the solution was stripped to yield 1.55 g product (4 % grafting).

3. Scale-Up of Synthesis and Purification

Scale up Reaction to Graft Poly(ethyloxazoline) DP= 30 on Poly(ethyleneimine) DP= 100:
Methyl tosylate (15.43 g, 83 mmol) was added to a 2 L round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubbler, followed by 1100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 100 ^{o}C, and then 2-ethyl-2-oxazoline (242 g, 2.24 mol) was added to the reaction vessel. The reaction vessel was gently refluxed (~110 ^{o}C) , and reaction was kept at this temperature for 24 hours. In a separate 250 mL round bottom flask was added poly(ethyleneimine) DP=100 (7.2 g, 167 mmol NH) and 150 mL toluene. The mixture was dissolved and dried by refluxing the toluene to a Dean – Stark trap for 30 minutes. This resulting hot solution was poured into the PEOX DP= 30 mixture cooled at ~100 ^{0}C, and reaction was gently refluxed under N_2 for 24 hours. Reaction vessel was cooled to room temperature and the volatiles were removed by rotary evaporator followed by high vacuum at 85 ^{0}C to give 216 g of crude product. This mixture was split into two equal portion of 108 g crude material. Each portion was dissolved in 1500 mL deionized water and ultrafiltered on a tangential flow ultrafiltration device at 25 psi over 30K regenerate cellulose membranes to give 5 recirculations of permeate. The retentate was stripped of volatiles on a rotary evaporator followed by high vacuum at 85 ^{0}C to give 48.8 g of G= 1 dendrigraft product that was clean of unreacted PEOX DP= 30 by SEC. The second half of the crude material gave 52 g product under the same conditions to give a total of 101 g product.

Reduce Amount of Reagents
The propargyl tosylate initiated PEOX was analyzed for acetylene content. There has been a question as to whether each PEOX chain was initiated by propargyl tosylate or a mixture of propargyl tosylate and other initiators. Benzyl azide was prepared and reacted with a Cu(I) catalyzed click reaction with PEOX DP = 10 and 30. A ^{1}H NMR spectrum of this mixture indicated that all the PEOX chains contained a propargyl group by integration of the triazole proton vs the morpholine protons (the living end of the propargyl tosylate initiated PEOX was quenched with morpholine giving a morpholine group on one end and a propargyl group on the other.

The preparation of the azide was investigated using PEI and morpholine as model compound. The reiterative reaction of epichlorohydrin and sodium azide was worked out with PEI

initially. The disappearance of epichlorohydrin was found to be easily monitored by adding a small aliquot to iodine in methanol and running a TLC in methanol. Any spot at $R_f \sim 0.8$ on the TLC plate indicates a epichlorohydrin – iodine adduct. Morpholine was reacted with one equivalent epichlorohydrin in methanol at room temperature for about 4 hours followed by sodium azide at 65 ^{0}C in methanol. This was the same procedure developed for the derivatization of PEI. This reaction clearly produced the desired product and a small amount of the chlorohydroxypropyl azide as determined by ESI – mass spectroscopy and ^{13}C NMR spectroscopy.

In the experiments describing the catalyzed click reaction, the copper was generated from a reduction of copper sulfate with sodium ascorbate or from small pieces of copper wire that were stirred with the reaction mixture. It appears that the copper works well releasing a small amount of copper into the system. These catalyzed click reactions demonstrated that the reaction does occur with the use of 100 %, 50 % and 25 % Cu(I) catalyst per nitrogen of the PEI or PEOX – PEI core using the copper sulfate – sodium ascorbate system. No further reduction in catalyst was examined at that time. The reaction also occurs with a few percent of copper released into solution with copper wire.

The common observation of each reaction was that the yield was very low, ~ 1- 2 %, as determined with isolation by ultrafiltration. All other SECs of final product mixtures, thermal and catalytic, were approximately the same. The conclusion from this set of experiments is that the actual azide content was much lower than thought. The reaction to prepare the hydroxyproylazide was not quantified on the PEI or PEOX – PEI core. The azide absorption coefficient in the infrared spectrum is high relative to the other frequencies and distorts the perceived alkyl azide content.

The thermal reaction of the PEOX DP = 10 and PEI functionalized with azide was examined first and found to react at 125 ^{0}C in refluxing n – butanol. The yield was low because of the low azide content. The reaction was done with a longer PEOX DP = 30 and was found to be successful under the same conditions. The reaction was explored further by refluxing in water for 18 hours to give the same yield. This reaction in water at 75 ^{0}C for 18 hours was also successful but at a little lower yield than at 100 ^{0}C. This suggests that the optimum reaction temperature for this particular set of substrates was above 75 ^{0}C.

Scale up Reaction Optimization with Click Chemistry: Scale up of G1(200-60) PEI Dendrigraft Synthesis using Click Reaction:

Preparation of Propargyl Tosylate-Initiated Polyethyloxazoline, DP = 60: Propargyl tosylate (530 mg, 2.50 mmol) was added to a 250 ml round-bottom flask, fitted with a Dean-Stark apparatus, a condenser, and a nitrogen bubble, followed by 100 mL of toluene. The reaction vessel was heated to reflux for 30 minutes, and then water was removed by azeotropic distillation with toluene through the Dean-Stark trap. After complete removal of water, the reaction vessel was allowed to cool to 50 ºC, and then 2-ethyl-2-oxazoline (4.96 g, 300 mmol) was added to the reaction vessel. The reaction vessel was heated to 110 ºC, and reaction was kept at this

temperature for 18 hours. Morpholine (0.09 g, 1.0 mmol) was added quickly, and reaction was continued under same condition for another 18 hours. Reaction vessel was cooled down to room temperature, and then solvent was removed under reduce pressure to yield product (brown solid).

Preparation of Chlorohydroxypropyl Azide: To a 500 mL round bottom flask was added sodium azide (52 g, 800 mmol, 5 equivalents), 250 ml deionized water and acetic acid (147 mL, 154 g, 2.57 moles). To this homogeneous mixture was added epichlorohydrin (14.8 g, 160 mmoles). This mixture was stirred at 300 ºC for 5 hours. To this resulting mixture was added $NaHCO_3$ until the solution until gas evolution ceased. Sodium chloride was added until the solution was saturated. This mixture was extracted with 3x150 mL diethyl ether. The combined organic layers were washed with $NaHCO_3$ solution and brine and dried with sodium sulfate. The ether was removed on a rotary evaporator at 300 mmHg then 740 mmHg for about 10 minutes to give 21 g (95 % yield).

Preparation of Azidopropylepoxide: To a 250 mL round bottom flask containing a stir bar was added sodium hydroxide (7.08 g, 177 mmol, 1 equivalent) and 100 mL deionized water. This solution was made homogeneous and allowed to cool to room temperature. To this well stirred mixture was added all at once chlorohydroxypropylazide (24 g, 177 mmol). This mixture was stirred for 5 minutes at room temperature. Sodium chloride was added to this mixture to saturation. This mixture was extracted with 3x70 mL diethyl ether and the combined organic layers dried over sodium sulfate. The filtered solvent was stripped of volatiles at 300 mm Hg on a rotary evaporator with the water bath at 25 ºC followed by a final evacuation for about 10 – 15 minutes at 740 mmHg to give 17 g (95 % yield) of the title compound containing ~ 5 % ether by ^{1}H NMR spectroscopy.

Preparation of PEI DP = 200 Hydroxypropylazide 50 % Azide: To a 250 mL round bottom flask containing a stir bar was added polyethyleneimine DP =200 (10g, 1.2 mmol, 233 mmol NH) and 100 mL MeOH. To this homogeneous mixture was added azidopropylazide (17g, 175 mmol) and the resulting mixture stirred at 35 ºC for 24 hours under N_2. The volatiles of this mixture were removed by rotary evaporator followed by evacuation at high vacuum with a bulb to bulb distillation apparatus at 100 ºC for 30 minutes to give 21.5 g. A ^{1}H NMR spectrum of this material indicated the hydroxypropylazide content was 50 % by integration.

Reaction of PEI DP = 200 Hydroxypropylazide (50 % Azide) with Propargyl initiated PEOX DP = 60 with 12 % Copper Catalyst: To a 1 L round bottom flask with a stir bar and fitted with a N_2 bubbler was added PEI DP = 200 hydroxypropylazide (50 % azide) (10 g, 109 mmol) , propargyl tosylate-initiated PEOX (DP = 60) (261g, 43.6 mmol) and 200 mL MeOH. This mixture was made homogeneous and brought to a pH of 3 using aqueous HCl monitoring with a pH meter. To this mixture was added 160 mL of deionized water and sodium ascorbate (2.6g, 13.1 mmol). This mixture was stirred for 10 minutes and copper (II) sulfate (3.3 g , 13.1 mmol) dissolved in 160 mL of water was added all at once. Reaction was stirred at room temperature for 18 hours. A SEC of this crude product indicated a dendrigraft molecular weight of 140,000 for a product with 20 grafted PEOX polymers from a theoretical of 50. To this mixture was added 70 g Amberlite IRC – 748 ion exchange resin and the resulting mixture stirred for 20 hours at room temperature. This mixture was filtered of resin using a Buchner funnel with fast flow filter paper.

The filtered solution was stripped of volatiles on a rotary evaporator to give 280 g of crude material.

Hydrolysis of G1(200 PEI- 60 PEOX) Dendrigraft to G1(200-60): To a 1000 mL round bottom flask containing a stir bar and fitted with a Dean – Stark trap and a condenser was added G1 dendrigraft, PEI DP = 200 core, PEOX DP = 60 branch (50 g, 504 mmole PEOX repeat unit) and 53 mL sulfuric acid premixed with 530 mL deionized water. This mixture was refluxed to remove water and propionic acid azeotrope while replacing an equal volume of water. This process was repeated to remove 500 mL distillate. This resulting mixture was refluxed for 26 hours, cooled to room temperature and adjusted to pH 12 with sodium hydroxide. From this mixture was distilled ~ 200 mL water and 300 mL toluene added. The remaining water was azeotroped from the mixture while adding an equivalent volume of toluene. When all the water was removed, the hot toluene was filtered through a Buchner funnel with fast flow filter paper. The toluene was stripped on a rotary evaporator followed by a bulb to bulb distillation at high vacuum at 100 ºC for 45 minutes to give 18 g (90 % yield).

Preparation of G1(200-60)-TMAC: To a 500 mL round bottom flask with a stir bar was added dendrigraft G1(200-60) (10.25 g, 238 mmol NH) and glycidyl TMAC (16 g, 1106 mmol, 43 % per NH) and 180 mL MeOH. This mixture was refluxed for 16 hours. The volatiles were removed by rotary evaporator and the resulting residue dissolved in 400 mL deionized water. This mixture was stripped of volatiles to give 26 g (43 % grafting.

Preparation of Crosslinked G1(200-60)-TMAC: To a 25 mL two neck round bottom flask fitted with a mechanical stirrer and a N_2 bubbler was added dendrigraft G1(200-60) functionalized with 43 % glycidyl TMAC (5 g) dissolved in 28 g isopropanol (15 wt % solution). To this mixture was added poly(ethylene glycol) diglycidyl ether (MW = 252) (750 mg, 15 wt %). This resulting mixture was heated with stirring for 1.5 - 2 hours during which the viscous liquid changed to a solid product.

<u>Crosslink Dendrigrafts without Removing Excess PEI by Ultrafiltration</u>

The solution of living end PEOX (DP60, 25.55 g, 0.2578 mmoles) was added into PEI (DP = 200, 0.46 g, 10.69 mmoles) to form dendrigraft with some free PEOX. Free PEOX was not removed through ultra filtration. This mixture was hydrolyzed to form PEI. Four grams of this PEI sample was refluxed with glycidyl TMAC (6.32 g) to yield 9.73 g of G1(200-60)-TMAC. Five grams of this G1(200-60)-TMAC sample was cross-linked using following conditions: cross-linker: 1.5 g, 30%; isopropanol: 56.6 g, temperature: 80 ^{o}C; time: 5 hours. Total capacity test was carried out with this sample to know its binding capacity for perchlorate, nitrate and sulfate.

4. Materials' stability and physicochemical properties

Nuclear Magnetic Resonance (NMR) Spectroscopy:
Sample preparation: To 50-100 mg of a dry sample was add 800-900 μL of a deuterated solvent to dissolve. Typical reference standards are used, *i.e.*, trimethylsilane. Typical solvents are $CDCl_3$, CD_3OD, D_2O, DMSO-d_6, and acetone-d_6. The dissolved sample was transferred to an NMR tube to a height of ~ 5.5 cm in the tube. Equipment: (1) 300MHz NMR data were obtained on a 300MHz 2-channel Varian™ Mercury Plus NMR spectrometer system using an Automation Triple Resonance Broadband (ATB) probe, H/X (where X is tunable from ^{15}N to ^{31}P). Data acquisition was obtained on a Sun Blade™ 150 computer with a Solaris™ 9 operating system. The software used was VNMR v6.1C. (2) 500MHz NMR data were obtained on a 500MHz 3-channel Varian™ Inova 500MHz NMR spectrometer system using a Switchable probe, H/X (X is tunable from ^{15}N to ^{31}P). Data acquisition was obtained on a Sun Blade™ 150 computer with a Solaris™ 9 operating system. The software used was VNMR v6.1C.

Infrared (IR) Spectroscopy:
Infrared spectral data were obtained on a Nicolet Fourier™ Transform Infrared Spectrometer, Model G Series Omnic, System 20 DXB. Samples were run neat using potassium bromide salt plates (Aldrich).

Matrix-Associated Laser Desorption Ionization-Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS):
Mass spectra were obtained on a Bruker Autoflex™ LRF MALDI-TOF mass spectrometer with Pulsed Ion Extraction. Mass ranges below 20 kDa were acquired in the reflector mode using a 19 kV sample voltage and 20 kV reflector voltage. Polyethylene oxide was used for calibration. Higher mass ranges were acquired in the linear mode using a 20 kV sample voltage. The higher mass ranges were calibrated with bovine serum albumin. Typically, samples were prepared by combining a 1 μl aliquot of a 5 mg/ml solution of the analyte with 10 μl of matrix solution. Unless otherwise noted, the matrix solution was 10 mg/mL of 2,5-dihydroxybenzoic acid in 3:7 acetonitrile:water. Aliquots (2 μl) of the sample/matrix solution were spotted on the target plate and allowed to air dry at RT.

Size Exclusion Chromatography:
Dendrigraft samples were reconstituted with the mobile phase used in the SEC experiment (5 mg/ml concentration). All the samples were prepared fresh and used immediately for SEC. Samples were analyzed qualitatively by the SEC system (Waters 1515) operated in an isocratic mode with refractive index detector (Waters 2414 and Waters 717 Plus Auto Sampler). The analysis was performed at RT on Waters Ultrahydrogel coloumn (7.8 ID X 300 mm). The mobile phase of acetate buffer (0.5M) was pumped at a flow rate of 0.5 ml/min and chromatograph was analyzed by Waters Breeze software

Porosimeter:
BET Surface area and porosity of resins were analyzed using Micrometrics ASAP 2020 analyzer. 2.0 g of sample were degassed for 4 hrs, then N_2 gas adsorption was measured in duplicates using a temperature ramp of 1 ºC/min.

Atomic Force Microscopy (AFM)

AFM was performed on a Veeco Pico Plus atomic force microscope. G1(200-60)-TMAC was added to mica and dried. All images were obtained with a Pico-SPM™ LE AFM (Molecular Imaging, USA) in DI water with tapping mode, using Multi-purpose large scanner and MAC mode Tips [Type II MAClevers, thickness: 3 μm, length: 225 μm, width: 28 μm, resonance frequency: ca 45 KHz and force constant: ca 2.8 N/m (Molecular Imaging, USA)]. Typically, 3 lines/sec. scan speed was used for scanning different areas, with a set point of 0.90 of the cantilever oscillation amplitude in free status. To avoid hydrodynamic effect of thin air gaps, the resonance was carefully measured at a small tip - sample distance.

Biological Stability and Toxicity:

To perform the assay, Caco 2 cells were plated in 96-well cell culture plates at 30,000 cells/well, in 100μl cell culture media (10% fetal bovine serum/modified Eagle's medium). When the cells were ~75% confluent, the media was aspirated and lyophilized dendrimer resuspended in cell culture media was added to the cells at a range of concentrations (0.1-500 μg/well) in a final volume of 100 μl/well. The cells were incubated with the dendrimers for 20hrs at 37 ºC and 5%/CO_2, at which time 20μl of a 5mg/mL MTT solution was added to each well. The cells were allowed to incubate another 4hrs before the media was aspirated and replaced with 200μl DMSO. The 96-well plates were then quantified spectrophotometrically at 570 and 690 nm on a Multiskan MCC/340 microplate reader (ThermoLabsystems). Samples were run in triplicate for each concentration tested.

5. Total Capacity

Sample preparation for UF measurements for soluble resins:

All sodium perchlorate and dendrigraft solutions were prepared in DI water. A solution of dendrigraft (1g/50 mL) prepared in DI water and combined with a solution of $NaClO_4$ (50mL, 2ppm) to give 100 mL of 1ppm perchlorate-dendrigraft solution. This solution was stirred at RT for 1h and then filtered through Ultrafiltration membranes (two 10 kDa regenerated cellulose membranes, 4 recirculation's collecting 100 mL of permeate). A sample of retentate (2 mL) and permeate (2 mL) was collected after each recirculation. During ultrafiltration the volume of retentate was kept constant by adding DI water. A total of 4 permeate and 4 retentate samples were collected from each experiment. And, 10 ppm, 100 ppm, 1,000 ppm and 10,000 ppm sodium perchlorate and dendrigraft (G1-TMAC, G1-TEAC and G1-TBAC) samples were prepared by following the same experimental procedure.

Sample preparation for total capacity measurements for insoluble resins:

A. Moisture-holding capacity (MHC) measurements and quaternization of the resin:

1. 30 mL of DIUF (deionized-ultrafiltered) water is added to 0.5-1 g of resin. The resin is kept in water for 120 min.
2. The swallowed resin is de-watered under vacuum in a Buchner funnel (5 minutes) while washing several times with DIUF water.

3. 4.0±0.1 g of de-watered resin is weighed and recorded as W_{moist}, hydroxyl resin.
4. The W_{moist} resin is kept in 20 mL of 1 M HCl for 30 min. Then washed with water in a Buchner funnel and de-watered under vacuum for 5 min.
5. 10 mL of 1 M NaCl is passed through the resin. The effluent is collected in a vial and labeled as chloride resin.
6. The sample is dried through lyophilization and the weight is recorded as W_{dry}, chloride.

B. Total Capacity measurements for perchlorate, sulfate, and nitrate:

1. The W_{dry} chloride resin is kept in 20 mL of DIUF water for 30 min.
2. The swallowed resin is washed and de-watered under vacuum for 5 min.
3. 3.0±0.01 g of de-watered resin is weighed and recorded as W_{moist}, 2nd hydroxyl.
4. The W_{moist}, 2nd hydroxyl resin is taken in a Crucible Filter (coarse) and 10 mL of 0.001 M $NaClO_4$ (99 ppm ClO_4^-) solution is passed through it slowly. The effluent is collected in a vial and labeled as $NaClO_4$ effluent.
5. The resin is washed with 50 mL of 1 M NaCl solution and then washed with DIUF water for regeneration (regenerated resin 1).
6. 10 mL of 0.001 M Na_2SO_4 (96 ppm SO_4^{-2}) solution is passed through the regenerated resin 1. The effluent is collected and labeled as Na_2SO_4 effluent.
7. The resin is again washed with 50 mL of 1 M NaCl solution, and then washed with DIUF water for regeneration (regenerated resin 2).
8. 10 mL of 0.001 M $NaNO_3$ (62 ppm NO_3^-) solution is passed through the regenerated resin 2. The effluent is collected and labeled as $NaNO_3$ effluent.
9. The resin is collected and labeled.
10. All the effluents collected were analyzed by IC to determine the chloride, perchlorate, sulfate and nitrate retention.

Measurement of perchlorate using perchlorate ion selective electrode (ISE):
Perchlorate measurements were performed by using a Metrohm mV meter, which is a fully computer-controlled instrument. The measurements were done at room temperature. The pHoenix ISE was calibrated using standard solutions of sodium perchlorate of 1000 ppm, 100 ppm, 10 ppm and 1 ppm. A 2% (v/v) solution of 2M ammonium sulfate was added as an ionic strength adjustor and samples were mixed thoroughly by vortexing. Electrode slope check was done following the regulation of the pHoenix Electrode Co. and it was found within the range of 56 ± 2 mv. Then 2% (v/v) of 2M ammonium sulfate was added, the sample was vortexed and measured.

Measurement of perchlorate, nitrate, sulfate, and chloride using ion chromatography:
Ion chromatography was performed on a Dionex ICS100 system with CD25 conductivity detector, AS40 autosampler, analytical AS16 anion column (#055376), ASRS 4mm anion suppressor (#064554), and AS16anion guard column (#056899). Ion were eluted with 35mM NaOH, with 1.5 ml/min flow rate, and suppressor current at 130mA. Calibration graph was prepared for perchlorate, nitrate, and sulfate and chloride anions using Ion chromatography.

6. Adsorption Isotherm and Kinetic Measurements

Batch test at constant time (4 hrs) and variable perchlorate concentration with G1(100-30) and G2(100-30-15).

7. Operational Properties

Regeneration Efficiency
Cross-linked dendrigraft G1(200-60)-TMAC was used for this experiment. Total capacity protocol was used as discussed previously, except 0.1M NaCl was used for regeneration of dendrigraft (instead of 1M NaCl used before). Percentage binding was calculated from the source concentration after IC measurement instead of calculating gravimetrically. Anion solutions were passed through the fixed bed setup, without any dendrigraft, to identify if anions are passing through the fixed set-up without sticking (blank solutions).

8. Biofouling

Biofouling was determined by measuring the thickness of a layer of dendrigraft (200-60)-TMAC (crosslinked with poly(ethylene glycol)) on a silicon wafer. The silicon wafer was etched with 0.5M HF to increase adhesion of the dendrigraft film. The dendrigraft was swollen in water and heated at 100 ºC under continuous stirring, and the waver was dip coated with the solution. The film was dried under nitrogen. The substrate with thin film and an empty substrate were kept in 10 mL of 25 μg/mL albumin bovine solution in PBS buffer (pH 7) for specified times. The layer was washed with 10 mL of PBS buffer three times to remove unspecifically-bound BSA. The thickness was measured by an Angstrom PHE101 ellipsometer using the Cauchy model after 0, 6, 12, 18, and 24 hours.

Results and Discussion

1. Synthesis of Priostar® Dendrigraft Polymers

Dendrigraft polymer was synthesized from two different cores: a linear polyethyleneimine (PEI) core, and a small poly(vinylbenzyl chloride) (PVC) microsphere core. The PEI core has the advantage of maximum capacity; the complete volume of the particle can be functionalized with perchlorate binding sites. The PVC microsphere core brings physical strength into the PEI-core particles, and still a much larger volume of the particle will be used than existing surface-substituted ion exchange resins due to the large branched dendrigraft chains.

The two different synthesis methods discussed above are illustrated in Figure 12. For linear PEI cores branching was initiated from a long backbone, in PVC microsphere core case branching was initiated from a micro bead. Both syntheses were developed and are effective in obtaining the desired structures.

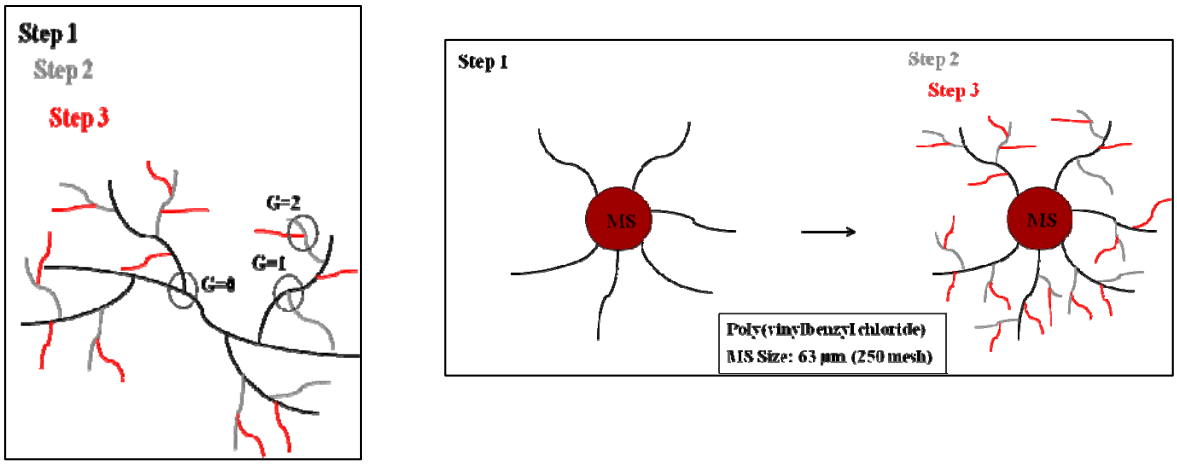


Figure 12. Two methods to synthesize PriostarTM dendrigrafts; A: branching from a linear backbone; B: branching from a PVC micro bead.

The advantage of the PEI core synthesis is that it maximizes active surface area; but branched resins are generally soft materials and might not withstand the pressure in a treatment system. Dendrigrafts were additionally strengthened by crosslinking with poly (ethylene glycol) chains. PEG has the additional advantage that it reduces binding of proteinaceous materials and thus biofouling. PVC microsphere core approach decreases the active surface area considerably, but strengthens the material due to the PVC bead.

Strategy 1: Synthesis of Functional Dendrigraft Polymers Based on Linear Poly (ethyleneimine), PEI Cores

Living-end Poly (oxazoline), PEOX grafting onto linear Poly (ethyleneimine), PEI cores followed by partial hydrolysis was carried out**.** Poly (oxazoline), PEOX chains of different lengths have been synthesized to serve as the core, or backbone, of the dendrigraft molecules. In general, backbone chains are long PEOX molecules, described as DP= 50, 100, and 200. PEOX chains are then hydrolyzed to form poly(ethyleneimine), PEI chains by removing the amide groups (see red circle in Figure 13), leaving free secondary amine groups for the subsequent grafting of PEOX side chains onto the backbones. Side chains are PEOX chains of lengths DP= 50, 30, and 15. The general approach to dendrigraft polymers is shown in Figure 12A, and the synthesis is shown in Figure 13.

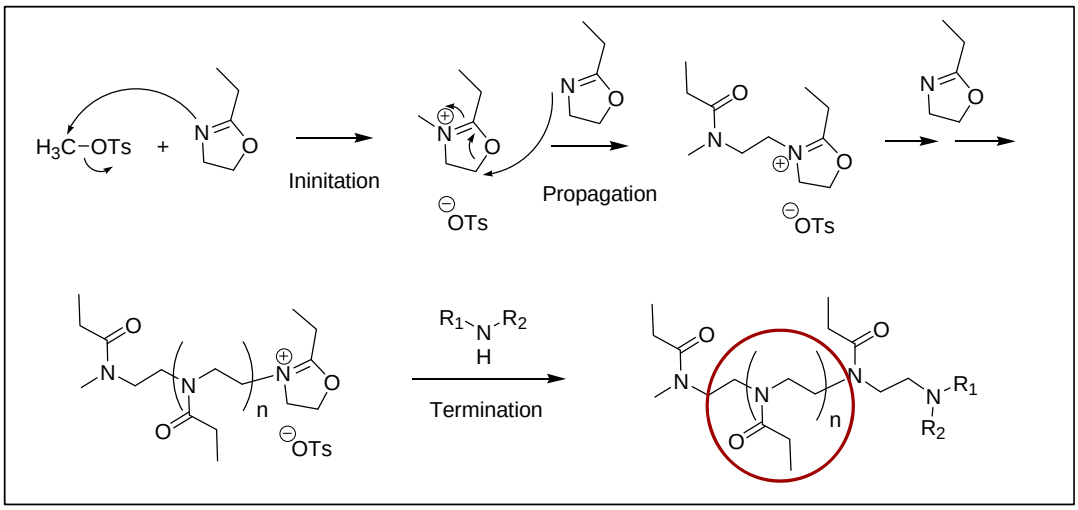


Figure 13. Methyl tosylate initiated living-end polymerization of 2-ethyl-2-oxazoline to form poly(oxazoline), PEOX chains of different lengths (DP= 50, 100, 200). Hydrolysis of amide groups (red circle) produces poly(ethyleneimine), PEI chains containing free secondary amines for grafting of side chains.

Poly(ethyleneimine) PEI Cores: The poly(ethyleneimine) cores produced for this study were DP= 50 and 100 prepared from polymerization of 2-ethyl-2-oxazoline. The PEOX polymers were characterized by ^{1}H-NMR spectroscopy, Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF MS) or Electron Spin Ionization Mass Spectroscopy (ESI-MS), and Size Exclusion Chromatography (SEC) to confirm the degree of polymerization before hydrolysis to PEI. This analysis sequence is shown below as an example for a PEOX DP= 50, in which the polymerization was terminated with morpholine (Figures 14-16).

In addition to DP= 50 and 100, a longer PEI core, DP= 200 was produced by two different procedures. The first method included propargyl tosylate initiated polymerization of 2-ethyl-2-oxazoline by heating the reaction mixture at 110^{o}C for 36 hours. SEC analysis indicated the formation of PEOX DP= 200. The polymer was hydrolyzed to PEI and a portion was converted back to PEOX polymer by reaction with propionyl chloride in pyridine to confirm its size. SEC showed a molecular weight of 15,000 Da instead of the theoretical 20,000 Da due to incomplete conversion. ^{1}H-NMR analysis indicated a mixture of 8% PEI and 33% PEOX, which accounted for the 5000 Da molecular shortfall. The second method included methyl tosylate initiated living-end polymerization of 2-ethyl-2-oxazoline in a dimethylformamide (DMF)/ toluene solvent mixture instead of the neat toluene used in the polymerizations of PEOX DP= 50 and 100.

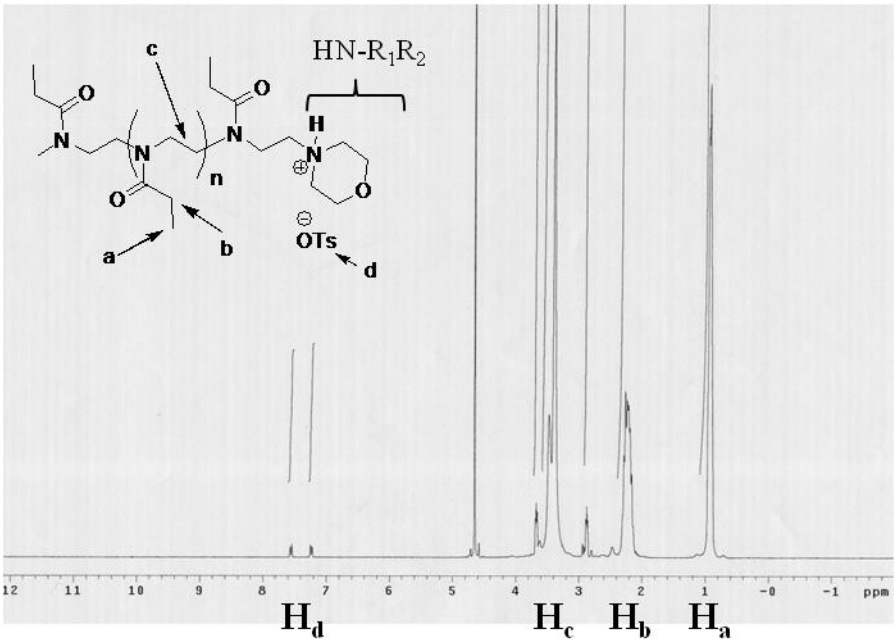


Figure 14. 1H-NMR spectrum of PEOX DP= 50 with peak assignments. The actual size of this polymer calculated by the integrals of the Ha and Hd protons equals DP= 52.5 (DP = 4 x Int(Ha) / (3 x Int(Hd)).

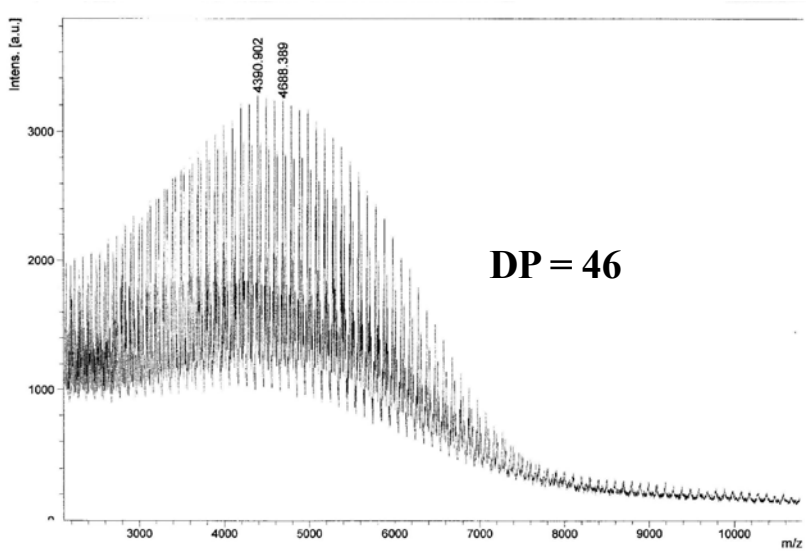


Figure 15. MALDI-TOF mass spectrum of PEOX DP= 50, giving an actual size of this polymer of DP= 46.

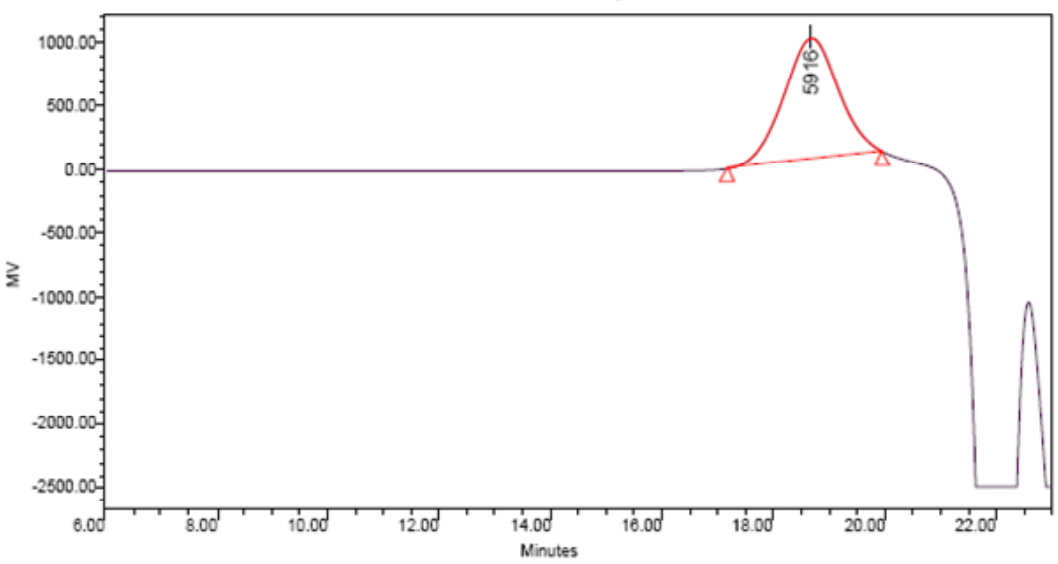


Figure 16. Size exclusion chromatogram of PEOX DP= 50, giving a polydispersity factor Mz/Mw= 1.37.

Exploratory Reactions to Prepare Dendrigraft Polymers G= 1: PEI cores of chain lengths DP= 50 and 100 were reacted with PEOX side chains of different lengths to evaluate the degree of side chain grafting onto the core and to study the physical behavior of these dendrigraft polymers G= 1 as well as approaches to purify the resulting products. Poly (ethyleneimine), PEI DP= 50 core was examined for grafting efficiency with PEOX DP= 15, 30 and 50, while PEI DP= 100 core was examined with PEOX DP= 15, 30 and 100. Generally, three mixing stoichiometries of living-end PEOX chains per secondary amine (NH) group of the core PEI were examined: 100, 50 and 20% PEOX compared to the total number of NH groups. SEC analysis of the crude products gave information about the molecular weight of the dendrigraft polymers, and therefore, the overall grafting efficiency. The results are as follows:

- PEI DP= 50 core reacted with 20% PEOX DP= 50: A dendrigraft product corresponding to molecular weight MW= 38,000 Da (~6 PEOX attached) was isolated (theoretical MW= 51,000 Da or 10 PEOX).
- PEI DP= 50 core reacted with 20% PEOX DP= 30: Very little product of MW ~15,000 Da was isolated (theoretical MW= 31,880).
- PEI DP= 50 core reacted with 50% PEOX DP= 30: A product with MW= 51,000 Da was isolated (theoretical MW= 76,475).
- PEI DP= 50 core reacted with 100% PEOX DP= 30: A product with MW= 68,000 Da was isolated (theoretical MW= 150,800).
- PEI DP= 50 core reacted with 20% PEOX DP= 15: A product with MW= 15,000 Da was isolated (theoretical MW= 14,870).
- PEI DP= 50 core reacted with 50% PEOX DP= 15: A product with MW= 24,000 Da was isolated (theoretical MW= 37,175).
- PEI DP= 50 core reacted with 100% PEOX DP= 15: A product with MW= 29,000 Da was isolated (theoretical MW= 74,350).

- PEI DP= 100 core reacted with 25% PEOX DP= 100: A product with MW= 105,000 Da was isolated, amounting to 10% grafting (10 of 100 NH substituted).
- PEI DP= 100 core reacted with 20% PEOX DP= 50: A product with MW= 124,000 Da was isolated, amounting to 12% grafting (12 of 100 NH substituted).
- PEI DP= 100 core reacted with 50% PEOX DP= 30: A product with MW= 112,000 Da was isolated, amounting to 37% grafting (37 of 100 NH substituted).

These results indicated that the grafting density was (1) increasing with the amount of PEOX added to the PEI cores, and (2) decreasing with increasing PEOX chain length. Longer side chains presumably resulted in low grafting density because of steric hindrance due to chain coiling.

Purification Studies using Ultrafiltration: Purification of the crude dendrigraft polymers by ultrafiltration (UF) using 1, 10, and 30 kDa cut-off size exclusion membranes was studied on some of the dendrigraft polymers G= 1. Ultrafiltration is an easy to scale operation that can be applied to large quantities of materials.

Crude mixtures containing dendrigraft G1 (PEI/PEOX) (50-15) and excess PEOX DP= 15 were found to be completely retained by 1 and 3 kDa membranes, resulting in total recovery of the crude material. A 10 kDa cut-off membrane was found to remove all PEOX DP= 15 during six circulations when maintaining the retentate solution at low (<1%) concentration. The dendrigraft polymer G1 (50-15) was retained to 100% by the 10 kDa membrane; however, the low retentate concentration and therefore large volume needed for this separation provides a challenge during scale-up of this product. Unfortunately, dendrigraft polymer G1(50-15) completely passed through a 30 kDa cut-off membrane, and the crude mixture was recovered without purification.

PEOX DP= 30 was found to be retained by 1, 3, and 10 kDa cut-off membranes but was found to readily pass through a 30 kDa membrane. Unfortunately, dendrigraft polymer G1 (50--30)was found to slowly leak through the 30 kDa membrane as well, resulting in poor separation of this mixture and low yields of this dendrigraft. When using a longer PEI core, e.g., dendrigraft G1 (100-30), excess PEOX DP= 30 was clearly separated by the 30 kDa membrane as shown in Figures 17-19.

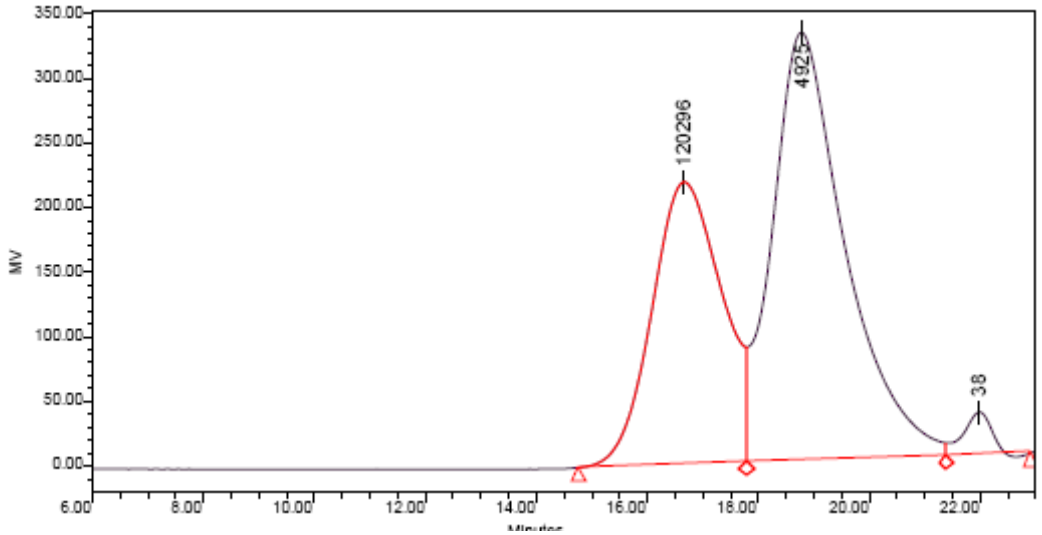


Figure 17. Size exclusion chromatogram of crude dendrigraft polymer G1 (PEI-PEOX)(100-30) mixed with excess PEOX DP= 30.

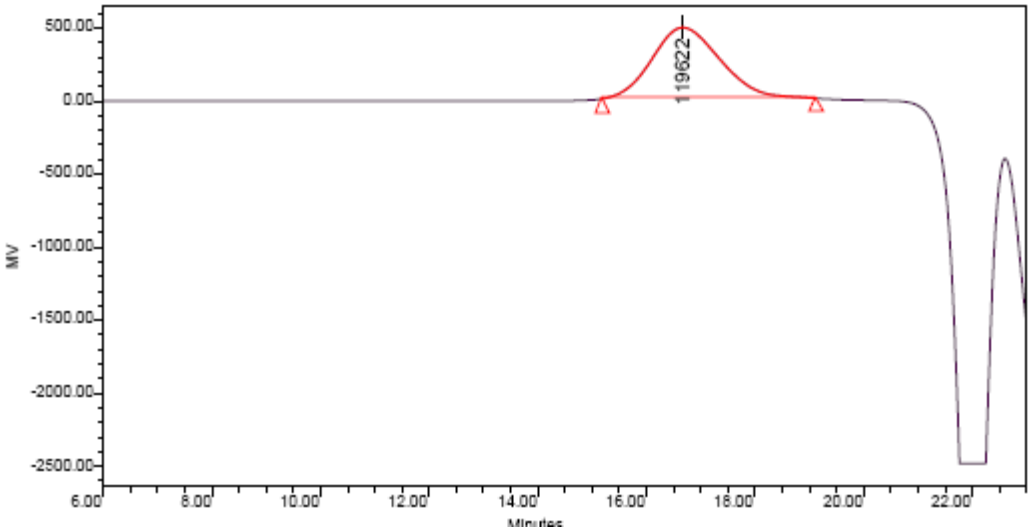


Figure 18. Size exclusion chromatogram of the retentate after ultrafiltration through 30 kDa cut-off membrane, containing dendrigraft polymer G1 (PEI/PEOX) (100-30). The negative signal in the chromatogram was caused by a change in the solvent composition.

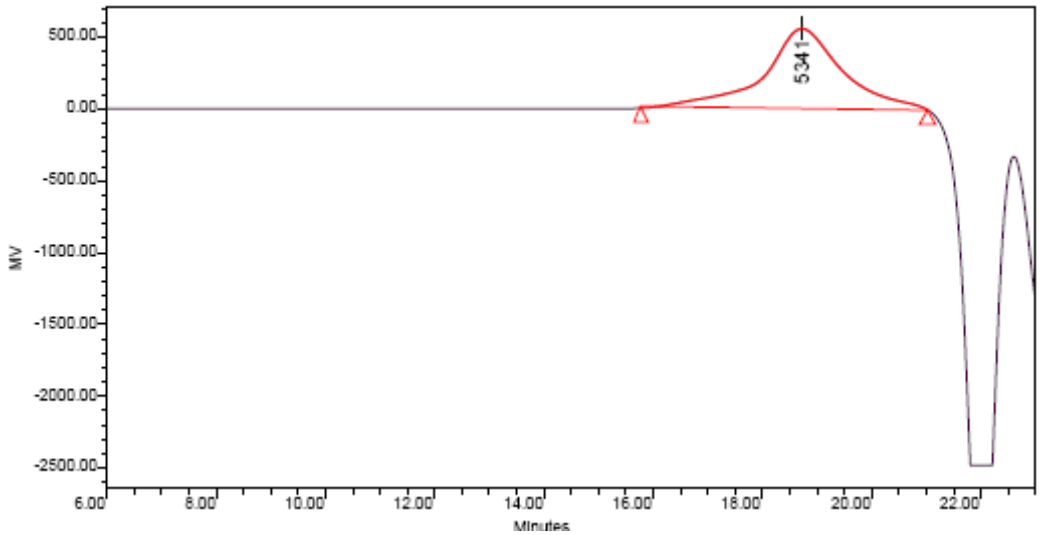


Figure 19. Size exclusion chromatogram of the permeate after ultrafiltration through 30 kDa cut-off membrane, containing excess PEOX DP= 30. The negative signal in the chromatogram was caused by a change in the solvent composition.

Partial Hydrolysis of PEOX Side Chains to PEOX-PEI Side Chains: After successful production and purification of dendrigraft polymers G= 1, the PEOX side chains were partially hydrolyzed to remove amide groups and free up secondary amine (NH) groups in order to prepare the product for conjugation of the next generation of side chains and, eventually, conjugation of the perchlorate binding sites onto the polymer. The following model studies were carried out to evaluate conditions resulting in the desired degree of hydrolysis. Dendrigraft polymer 100/30 served as the model compound, hydrolyzed with sulfuric acid in water.

Purified dendrigraft G1 (100-30) was treated with sulfuric acid in water for 6 hours at 100^{o}C using 0.5, 1.0 and 2.0 equivalents acid per PEOX repeat unit. Treatment with 0.5 equivalent sulfuric acid gave 45% hydrolysis, i.e., a dendrigraft G1(100-30) composed of 45% PEI and 55% PEOX. Treatment with 1.0 equivalent sulfuric acid gave 58% hydrolysis, i.e., a dendrigraft G1 (100-30) composed of 58% PEI and 42% PEOX. Finally, treatment with 2.0 equivalents sulfuric acid gave 88% hydrolysis, i.e., a dendrigraft 100-30 composed of 88% PEI and 12% PEOX. This material became insoluble in water upon adjustment of the pH to 10-11, which resulted in neutralization of the positive charged ammonium groups to neutral, and therefore less water soluble, secondary amine (NH) groups.

Synthesize Priostar® Dendritic Polymers in at Least One Other Distinct Size: Seven additional distinct sizes were synthesized for comparison. G1 and G2 dendrigrafts of different compositions were prepared in order to evaluate the influence of dendrigraft structure on side chain grafting efficiency. Three core sizes have been used: PEI 100, 200, and 700. Dendrigraft polymers G1 produced are G1 (PEI 100- PEOX I 30), G1 (PEI 200-PEOX 60), G1 (PEI 700-PEOX 200), and G1 (PEI 700-PEOX 100). Dendrigraft polymers G2 produced are G2 (PEI 100-PEI 30-PEOX 15), G2 (PEI 100-PEI 30- PEOX 100), and G2 (PEI 200-PEI 60- PEOX 30).

Characterize Priostar® Dendritic Polymers by Traditional Analytical Techniques: All original and seven additional sizes have been characterized by traditional analytical techniques. All dendrigraft polymers G1 and G2 as well as the PEOX/PEI cores were routinely characterized by proton NMR spectroscopy, mass spectroscopy, and size exclusion chromatography (SEC). Relevant data is provided in the experimental parts above.

Summary:

- To synthesize the backbone, poly(oxazoline), PEOX chains of different lengths (DP= 50, 100, 200), a methyl tosylate initiated living-end polymerization of 2-ethyl-2-oxazoline was performed.
- The amides were hydrolyzed with HCl to form the free secondary amine and thus poly(ethylene imine), PEI. Treatment with 2.0 equivalents sulfuric acid gave 88% hydrolysis, i.e., a dendrigraft 100/30 composed of 88% PEI and 12% PEOX. This material became insoluble in water upon adjustment of the pH to 10-11, which resulted in neutralization of the positive charged ammonium groups to neutral, and therefore less water soluble, secondary amine (NH) groups.
- The first generation is synthesized from the PEI backbone by repeating the same reaction: the free amine is used to initiate another PEOX polymerization, which is then hydrolyzed to PEI. This can be repeated as often as desired for higher generations.
- Several core lengths have been synthesized: DP=50, 100, 200, and 700 ("DP" stands for "degree of polymerization", i.e. this is the number of repeating units the polymer has).
- From these cores different length branches (DP= 100, 60, 30, 15) have been synthesized from their free amines.
- This has been repeated for a second generation.
- Overall it has been found that the branch length reduces the grafting density of the branch (probably due to steric hindrance).
- It was observed that with a DP=700 core it is possible to reach the particle size desired for the ion exchange column with each single polymer molecule.
- After preliminary screening it was decided to work with four different backbones in more detail: G1(100s-0), G1(200-60), G2(100-30-15), and G2(200-60-30) (G1 and G2 stand for generations, the numbers in the parentheses stand for the DP of the backbone, the first generation, and the second generation, respectively).
- Purification of the crude dendrigraft polymers was performed by ultrafiltration (UF) using 1, 10, and 30 kDa cut-off size exclusion membranes. Ultrafiltration was chosen because it is an easy to scale operation that can be applied to large quantities of materials.

Strategy 2: Synthesis of Surface Functional Dendrigraft Polymers Based on Poly(vinyl benzyl) chloride Microsphere Cores

Living-End Poly(oxazoline), PEOX Grafting onto Poly(vinyl benzyl) chloride Microspheres Followed by Partial Hydrolysis: Poly(oxazoline), PEOX chains of different lengths have been grafted onto the surface of poly(vinyl benzyl) chloride microspheres (63 μm or 250 mesh in size), which served as the cores of the dendrigraft molecules. This strategy has been investigated because the presence of microspheres should provide physical stability to the dendrigraft

polymers, rendering them insoluble in water as required for the final application. In addition, these microspheres should prevent compaction of the polymer during water filtration, resulting in reduced water flow which is a potential challenge to the corresponding dendrigraft polymers with linear PEI cores. The downsides of this strategy are (1) reduced active surface of the final dendrigraft because the microspheres cannot be penetrated by water and do not carry perchlorate binding sites within the spheres; these dendrigrafts are therefore more similar to current water remediation resins although the spheres are substantially smaller than current anionic exchange beads (300-1,200 μm or 16-50 mesh). (2) The presence of microspheres causes problems during analytic characterization of the dendrigraft polymers because microspheres do not dissolve in NMR solvents, and they interfere with SEC separations. Therefore, these dendrigraft polymers were mainly analyzed by infrared (IR) spectroscopy. The general approach to dendrigraft polymers with microsphere core is shown in Figure 20.

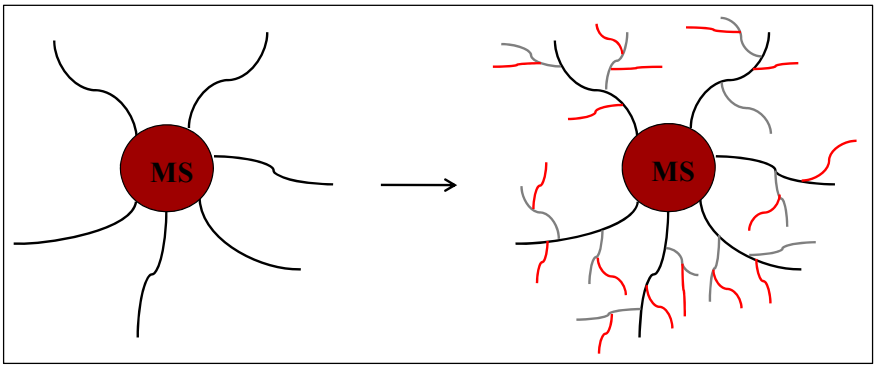


Figure 20. Schematic presentation of dendrigraft polymer formation: PEOX side chains are grafted onto a microsphere core, or backbone (MS). Then additional PEOX chains are grafted onto the first chains. Each branching point represents a generation G of the dendrigraft.

Surface Modification of Microspheres: The poly(vinyl benzyl) chloride microspheres used in this strategy were prepared following a published procedure[19]. However, the chloride surface needed to be converted into a secondary amine (NH) surface in order to apply the PEOX grafting protocol. Microspheres were therefore reacted with excess piperazine (PIPZ). Unfortunately, piperazine does not create strong vibration bands in IR spectra needed to monitor progress and completion of this reaction, and therefore, a portion of the modified microspheres was reacted with phenylisocyanate, taking advantage of the strong vibration band caused by the urea linkage formed in this reaction (Figures 21and 22). The appearance of the urea vibration at 1673 cm^{-1} indicated successful conversion of the chloride to the piperazine secondary amine (NH) surface; however, IR analysis will not provide information about the degree of conversion, i.e., whether the conversion was complete (100%).

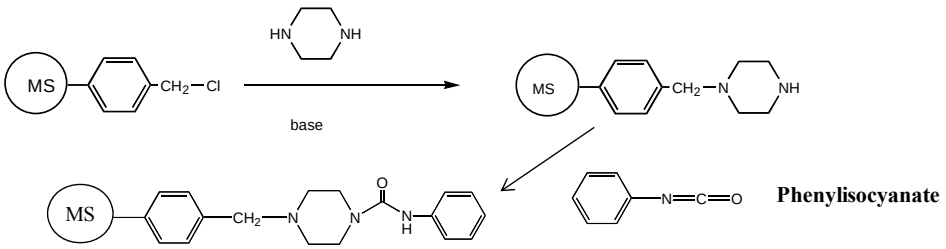


Figure 21. Reaction scheme showing the conversion of poly(vinyl benzyl) chloride microspheres to microspheres containing a piperazine secondary amine (NH) surface. A portion of microspheres with NH surface was derivatized with phenylisocyanate for improved infrared characterization.

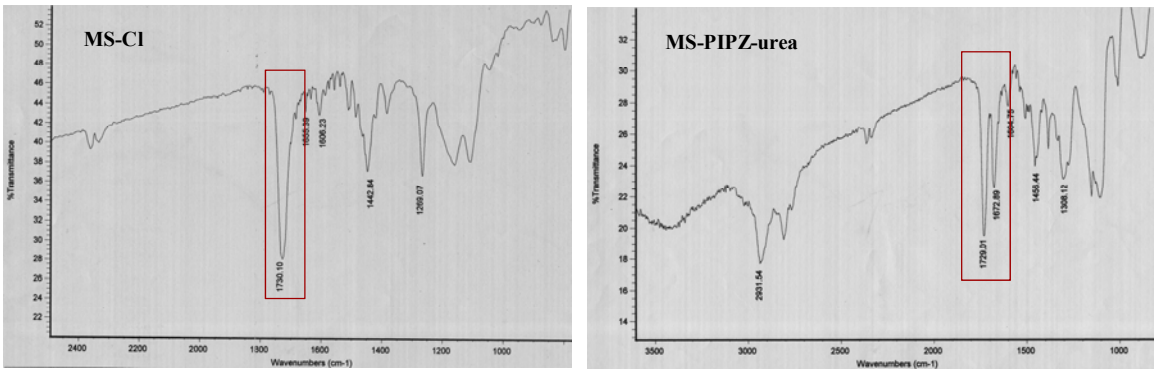


Figure 22. Left: IR spectrum of microspheres with chloride surface (MS-Cl), showing the ester vibration at 1730 cm-1, which is caused by ester groups within the microspheres and serves as the internal standard. Right: IR spectrum of piperazine microspheres, derivatized with phenylisocyanate (MS-PIPZ-urea). The urea linker causes the second strong vibration at 1673 cm^{-1}, indicating successful conversion of the microsphere surface.

Dendrigraft Polymers G= 0 with Microsphere Cores: Poly(vinylbenzyl) piperazine microspheres were reacted with PEOX DP= 20 chains as a model compound to produce dendrigraft polymer G=0. The reaction was monitored by IR spectroscopy, using the microsphere ester vibration at 1730 cm^{-1} as internal standard. A constant intensity ratio between ester vibration and amide vibration at 1648 cm^{-1} indicated that the PEOX grafting had been completed; however, IR analysis would not provide information about the degree of grafting, i.e., whether all NH groups had been consumed (Figure 23).

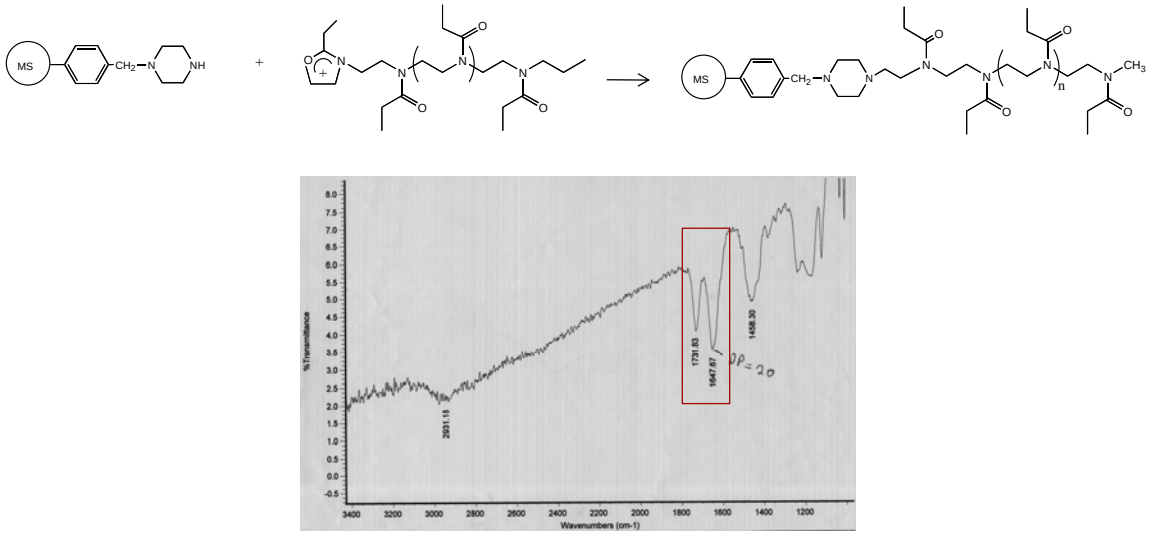


Figure 23. Reaction scheme of grafting PEOX DP= 20 onto the surface of microspheres MS-PIPZ; and IR spectrum monitoring the reaction through the intensity ratio of ester vibration at 1730 cm-1 and amide vibration at 1648 cm-1 (red box).

Dendrigraft Polymers G= 1 with Microsphere Cores: Dendrigraft polymer MS-PIPZ-PEOX G= 0 was partially hydrolyzed with sulfuric acid in water, transforming PEOX to PEI repeat units containing free NH groups as described earlier in this report. The resulting dendrigraft MS-PIPZ-PEOX-PEI G= 0 was reacted via living-end polymerization with 2-ethyl-2-oxazoline to produce dendrigraft polymer MS-PIPZ-PEOX G= 1. The reaction sequence was monitored by IR spectroscopy, using the ester vibration at 1733 cm^{-1} as internal standard: (1) Partial hydrolysis caused decreasing amide vibration at 1644 cm^{-1}, and (2) successful grafting of the next generation of PEOX chains caused increasing amide vibration at 1644 cm^{-1} (Figure 24). As mentioned before, IR analysis would not provide quantitative information about hydrolysis and grafting efficiencies; however, the changes in vibration ratios clearly gave proof that these reactions had occurred.

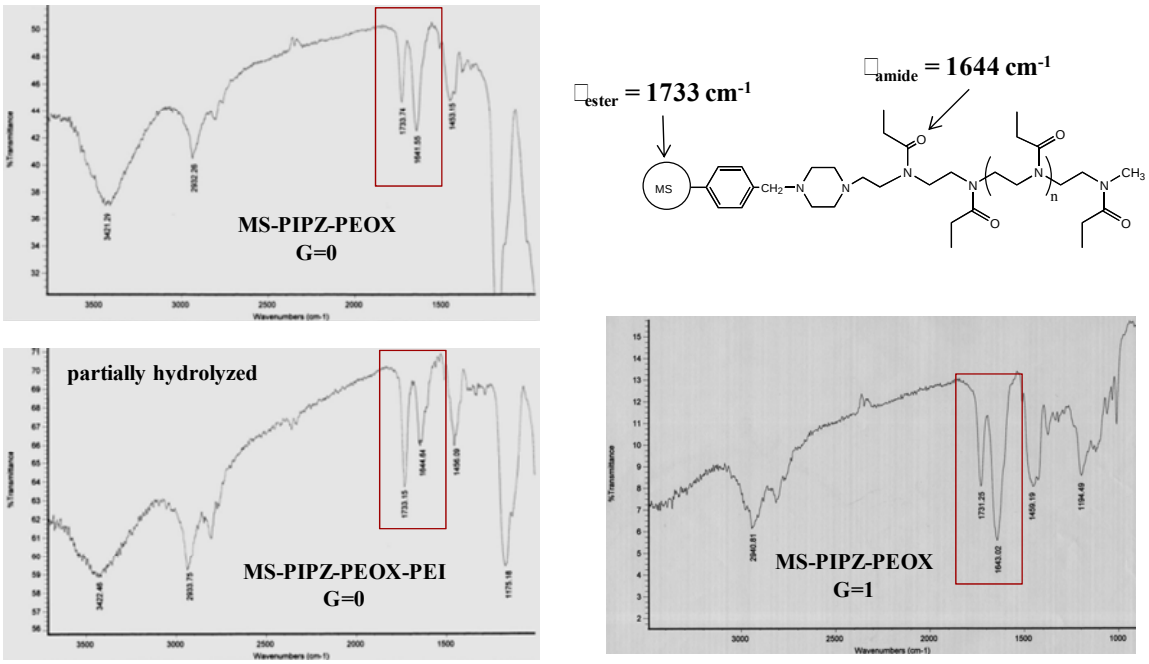
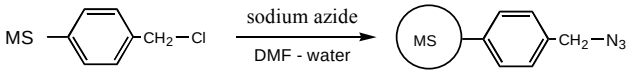


Figure 24. IR spectra-monitoring of partial hydrolysis of dendrigraft polymer MS-PIPZ-PEOX G= 0 to MS-PIPZ-PEOX-PEI G= 0, followed by living-end polymerization to produce dendrigraft MS-PIPZ-PEOX G= 1.

Transformation of Poly(vinyl benzyl) chloride Microspheres to Poly (vinyl benzyl) azide Microspheres Allowing Dendrigraft Formation Via Click Chemistry: Click chemistry, i.e., the copper(I) catalyzed reaction between azide functional groups (-N_3) and propargyl or acetylenic functional groups (-C≡C-), has become a major focus of research because of its fast and quantitative nature and the fact that the reaction has the potential to be conducted in water as an environmentally benign solvent[20]. In order to evaluate the applicability of click chemistry for the synthesis of dendrigraft polymers, poly(vinyl benzyl) chloride microspheres had to be converted



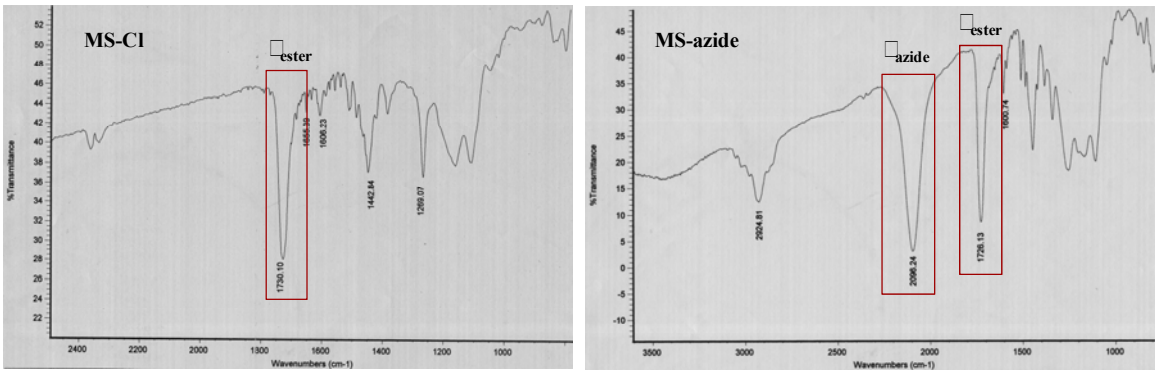


Figure 25. Reaction scheme of chloride-to-azide surface transformation of microspheres; and IR spectra monitoring the reaction through the appearance of azide vibration at 2096 cm-1 (red box). The ester vibration at 1730 cm-1 served as internal standard.

to microspheres with azide surface. This transformation was carried out by reaction of chloride microspheres with sodium azide in DMF-water solvent mixture. Progress of this reaction was monitored by IR spectroscopy, i.e., the appearance of the strong azide vibration at 2096 cm^{-1} (Figure 25).

Preparation of Propargyl-Terminated Poly(oxazoline), PEOX Chains: Grafting of PEOX chains onto azide microspheres via click chemistry required the preparation of propargyl (-C≡C-)-terminated PEOX chains. These chains were produced by propargyl tosylate initiated living-end polymerization of 2-ethyl-2-oxazoline. Three different chain lengths were produced, DP= 10, 50, and 100, as verified by MALDI-TOF mass spectroscopy and SEC analysis (Figure 26).

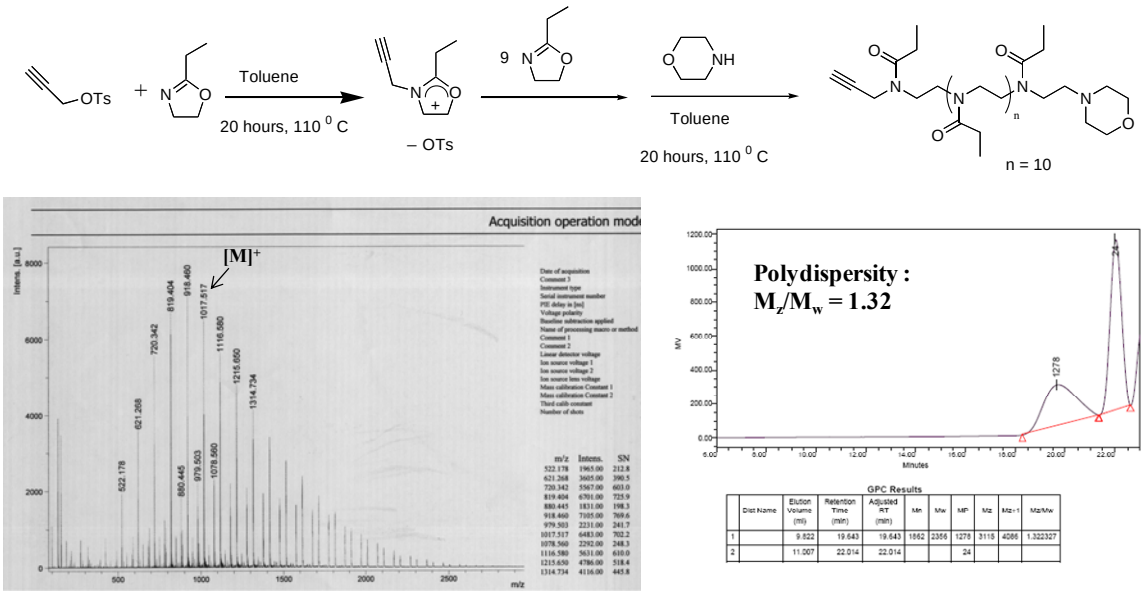


Figure 26. Reaction scheme of propargyl tosylate initiated polymerization of 2-ethyl-2-oxazoline to form propargyl-terminated PEOX chains (DP= 10); and MALDI-TOF mass spectroscopy and SEC analysis of the chains.

Dendrigraft Polymers G= 0 with Microsphere Cores via Click Chemistry: Azide microspheres were reacted with propargyl-terminated PEOX DP= 10 and 50 chains as model compounds to produce dendrigraft polymers G=0. PEOX chains were dissolved in DMF-water mixture, followed by addition of azide-terminated microspheres, aqueous copper(II) sulfate pentahydrate solution, and aqueous L-Ascorbic acid sodium salt solution. The resulting dendrigraft polymers with microsphere core were isolated by filtration and analyzed by IR spectroscopy, using the microsphere ester vibration around 1730 cm^{-1} as internal standard (Figure 26). The appearance of the amide vibration around 1648 cm^{-1} in both spectra indicated that the PEOX chains had been successfully grafted onto the azide surfaces; however, the different intensity ratios between ester and amide vibrations in both spectra could be the result of the different chain lengths and/or indicate different grafting densities. In any case, the remaining azide vibrations in both spectra around 2096 cm^{-1} gave clear evidence of incomplete grafting, leaving unreacted azide groups on the microsphere surfaces. Moreover, a comparison with the ester-*vs*-amide intensity ratio in the IR spectrum of dendrigraft polymer MS-PIPZ-PEOX (DP= 20) G= 0 shown in Figure 23

strongly suggested a lower grafting efficiency for the click chemistry approach: in Figure 23 the amide vibration is clearly stronger than the ester vibration, while in Figure 27 both amide vibrations are smaller than the ester vibrations.

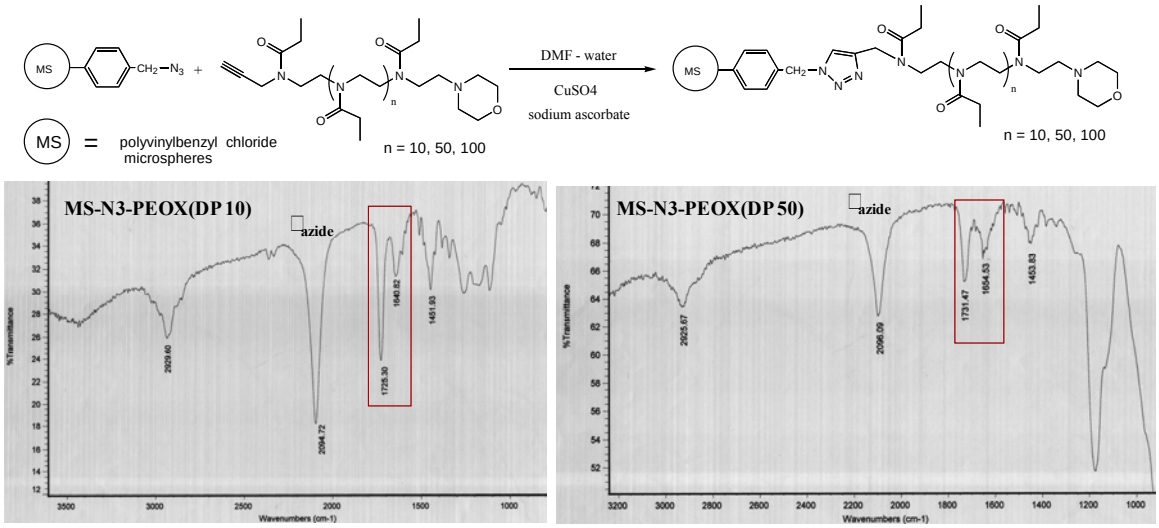


Figure 27. Reaction scheme showing grafting of propargyl-terminated PEOX DP= 10 and 50 onto azide microspheres to form dendrigraft polymers MS-N3-PEOX(DP= 10) and MS-N3-PEOX(DP= 50); and IR spectra monitoring the reactions. The appearance of amide vibrations around1648 cm^{-1} (red box) indicated successful grafting; however, the remaining presence of azide vibrations around 2096 cm^{-1} revealed incomplete reactions. The ester vibration around 1730 cm^{-1} served as internal standard.

The apparently lower grafting efficiency of the click approach together with scale-up challenges – few companies are prepared to scale azide-containing compounds, resulting in less competition, and therefore, likely higher cost of goods - and the analytical challenges of dendrigraft polymers with microsphere cores favor Strategy 1, the production of dendrigraft polymers with linear PEI cores.

Summary:

- Two methods were investigated to attach the branched polymers to the beads: 1) polymerization from piperazines (secondary amines) from the surface, and 2) "click chemistry" from azides on the surface. Grafting via click chemistry was incomplete; leaving some of these azides unreacted on the surface of the beads. Thus the first approach was selected for as a preferred route
- PVC beads were synthesized via radical polymerization according to a published procedure.
- Piperazine was grafted onto the surface via reaction of the bead with excess piperazine and base. Due to the beads the product cannot be characterized by NMR, and piperazine does not give a strong enough signal in IR spectroscopy. Thus piperazine was reacted phenylisocyanate, which showed a strong signal in the IR.
- To produce G0, piperazine microspheres were reacted with poly(ethylene oxazoline) (PEOX)). IR spectroscopy confirmed the reaction.
- Dendrigraft polymer MS-PIPZ-PEOX G= 0 was partially hydrolyzed with sulfuric acid in water, transforming PEOX to PEI repeat units containing free NH groups.

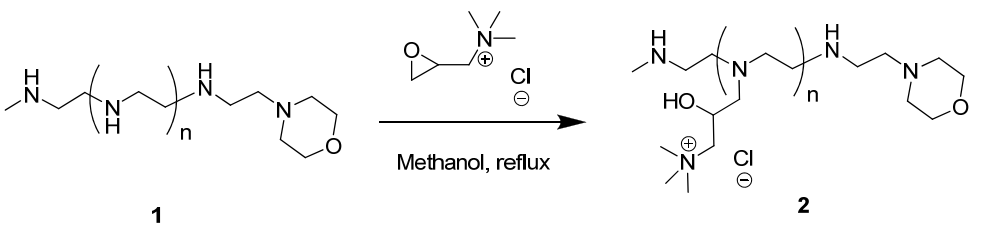
- The resulting dendrigraft MS-PIPZ-PEOX-PEI G= 0 was reacted via living-end polymerization with 2-ethyl-2-oxazoline to produce dendrigraft polymer MS-PIPZ-PEOX G= 1. The reaction sequence was monitored by IR spectroscopy and was shown to be successful.
- In conclusion due to likely higher cost of goods - and the analytical challenges of dendrigraft polymers with microsphere cores, production of dendrigraft polymers with linear PEI cores was selected for the further study.

2. Synthesize, Crosslink, and Characterize Perchlorate-Optimized Priostar® Dendrigraft Polymers

The most often used perchlorate binding site in commercial resins is comprised of tributyl ammonium groups grafted onto a polymeric carrier (e.g., ResinTech SIR-110-HP; Dow PSR-2, used by Siemens; CalRes 2109, used by Calgon; and PWA2, used by Rohm & Haas). Two strategies have been pursued: Strategy 1 contains the synthesis of trimethyl, triethyl, and tributyl ammonium sites grafted onto the dendrigraft polymers. While the perchlorate binding efficiency of these sites is likely comparable to the commercial binding site, the expected substantially higher number of binding sites per volume of polymer provides enhanced binding efficiencies of the dendrigraft polymers. The three different binding sites (methyl, ethyl, and butyl) were evaluated because of their size differences – a larger size could result in a lower grafting density due to steric hindrance, i.e., a larger binding site might not be able to penetrate the dendrigraft polymer completely. Strategy 2 contains the direct conversion of secondary amine (NH) groups throughout the dendrigraft polymers into quaternary ammonium groups, again using methyl, ethyl, and butyl groups. The advantage of this approach is the small sizes of the respective alkylating agents, leading to high grafting densities.

Strategy 1: Synthesis and Grafting of Quaternary Ammonium Cations onto PEI Chains

Reaction between Poly(ethyleneimine) and Glycidyl trimethylammonium chloride: Glycidyl trimethylammonium chloride is a widely used cationic agent for many applications and commercially available at an affordable price. This potential perchlorate binding site was reacted with PEOX DP= 100 as a model compound in methanol. ^{1}H-NMR and SEC analysis indicated successful grafting of the reagent onto the PEI backbone (Figure 28). The product showed well resolved NMR signals indicating a uniform product, an assumption supported by the low polydispersity factor of M_z/M_w= 1.14.



1 2

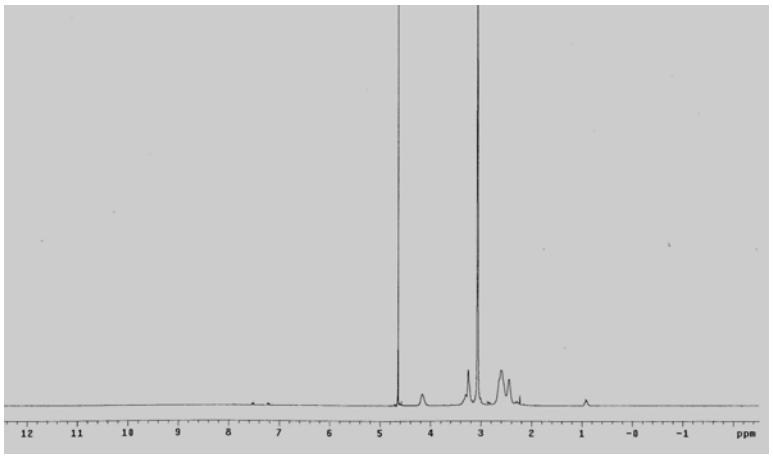
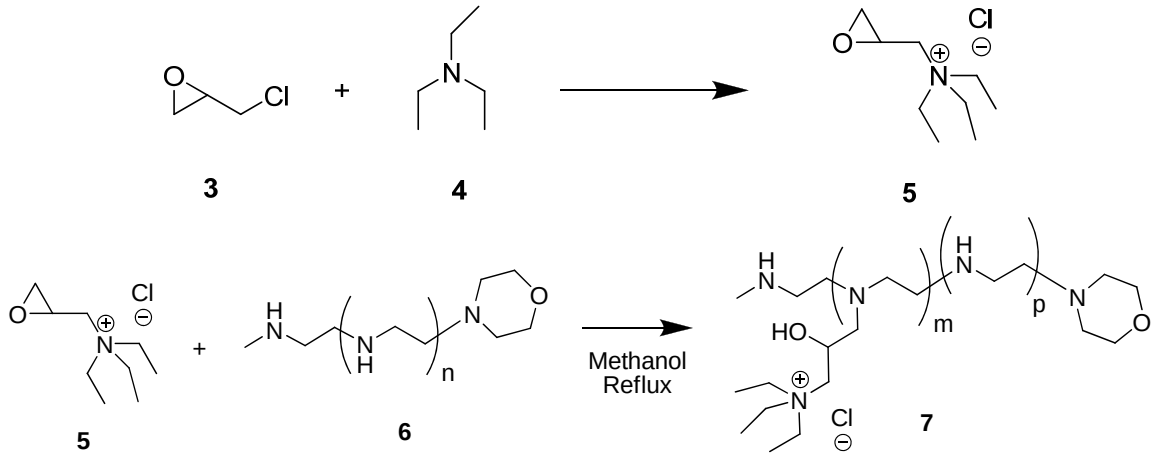


Figure 28. Reaction scheme showing grafting of glycidyl trimethylammonium chloride onto PEI DP= 100; and 1H-NMR spectrum showing very well resolved signals indicative of a uniform product.

Reaction between Poly(ethyleneimine) and Glycidyl triethylammonium chloride: Glycidyl triethylammonium chloride, the next potential binding site evaluated for PEI grafting, is not commercially available. It was prepared through reaction between epichlorohydrin **3** and triethylamine **4** (Figure 29). The reaction product was then reacted with PEI DP= 100 and characterized by ^{1}H-NMR and SEC, giving a polymer with low polydispersity factor of M_z/M_w= 1.25.



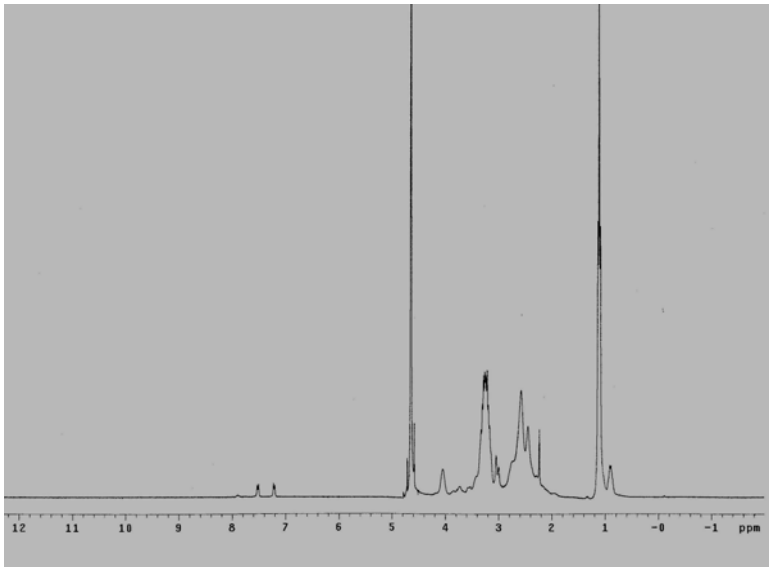
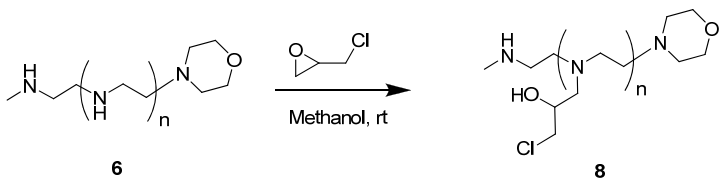


Figure 29. Reaction scheme showing the synthesis of glycidyl triethylammonium chloride 5 and its grafting onto PEI DP= 100; and ^{1}H-NMR spectrum showing well resolved signals indicative of a uniform product 7.

Reaction between Poly(ethyleneimine) and Epichlorohydrin: In the previous approach the binding site was first prepared and then grafted onto the polymer chain; however, this approach does not work for alkyl chains longer than ethyl because the reaction times increase significantly. Raising the reaction temperature is not an option due to the risk of prematurely opening the epoxy ring in the presence of a trialkylamine base and losing the ability to graft the product onto the polymer chain. In this new approach a linker molecule was first attached to the secondary amine (NH) groups of the polymer chain, and the ammonium binding site was then formed in a second reaction step. PEI DP= 100 was reacted with epichlorohydrin in methanol, forming polymer **8**, based on ^{1}H-NMR analysis (Figure 30). When compound **8** was dried and heated, the material became insoluble in all common solvents, including water, methanol, and chloroform. The polymeric material, however, absorbed large amounts of water, forming a hydrogel. This behavior could be explained by cross-linking of the polymer chains to form compound **9**. This cross-linking effect could become valuable later on in the project when water-soluble dendrigraft polymers need to be modified to render them water-insoluble. However, when compound **8** was heated with more epichlorohydrin in a solvent such as methanol, it continued to react with epichlorohydrin, forming soluble polymer **10**, as concluded from its ^{1}H NMR spectrum.



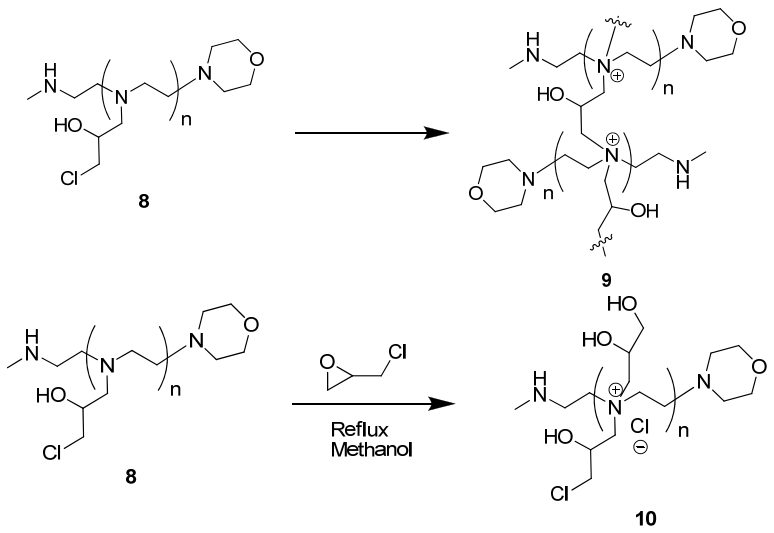
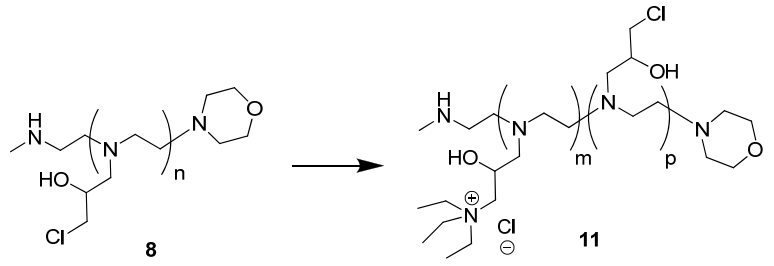


Figure 30. Reaction schemes showing products resulting from treatment of PEI= 100 with epichlorohydrin under different conditions.

Reaction between Epichlorohydrin-Modified Poly(ethyleneimine) and Triethylamine: Epichlorohydrin-modified PEI polymer **8** was treated with triethylamine in methanol in order to graft a potential perchlorate binding site onto the polymer. The resulting polymer **11** was purified by ultrafiltration and analyzed by ^{1}H-NMR spectroscopy. New NMR peak positions at 1.1 ppm suggested the presence of ethyl groups; however, the spectrum of **11** was significantly different from the spectrum of **7**, obtained from the direct reaction between PEI DP= 100 and pre-prepared glycidyl triethylammonium chloride (Figure 31).

To further elucidate the structure of **11**, PEI DP= 100 was reacted in a new approach simultaneously with a mixture (~1:1) of epichlorohydrin and glycidyl triethylammonium chloride. The NMR spectrum of product **12** was compared with the spectrum of polymer **11**. The highly similar NMR profiles indicated that the two-step approach actually resulted in a more complex backbone grafting pattern than anticipated, explaining the more complex NMR profile. Although the two-step approach introduced a serious amount of quaternary ammonium groups along the polymer backbone, regardless of the reaction sequence (i.e., whether the reactants were added sequentially or simultaneously), the direct reaction between PEI DP= 100 and glycidyl triethylammonium chloride gave the higher grafting density of ammonium groups.



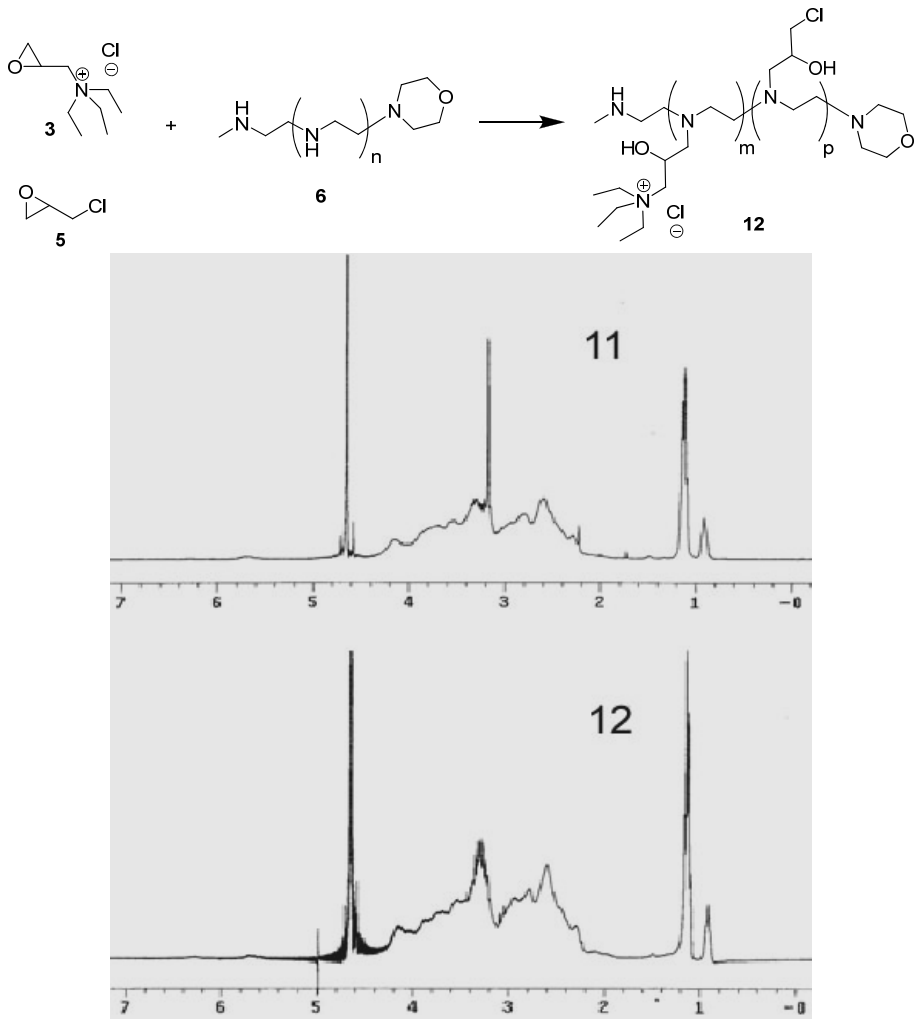


Figure 31. Reaction schemes showing the preparation of polymers 11 and 12; and 1H-NMR spectra of these polymers indicating identical structures of both polymers.

Strategy 2: Click approach to attach Quaternary Ammonium Cations onto PEI Chains:

The binding sites used will not be "clickable", but will be good perchlorate binding sites and will be easier to synthesize and add to the structure. Three binding sites have been synthesized and characterized by traditional analytical techniques. As described before, clickable binding sites did not lead to higher grafting efficiencies compared to the more classic reaction between secondary amine (NH) and epoxy rings. In addition, there are only a few scale-up facilities available that can handle and scale the click approach employing azide and C≡C triple bond moieties. Therefore, this route will not be further pursued, and emphasis will be on the development of perchlorate binding sites containing an epoxy ring as the reactive motif.

The third binding site of interest, glycidyl tributylammonium chloride was synthesized by reaction of epichlorohydrin and tributylamine, both of which are commercially available

inexpensive starting materials. Epichlorohydrin (22.7 g) and tributylamine (50 mL) were mixed in 200 mL of methanol in a 250-mL round bottom flask. The mixture was heated to reflux in an oil bath (~65^{o}C) for 3 hours and dried under high vacuum to yield the product in 39.4 g (58% of theory) as oil (Figure 32 and 33).

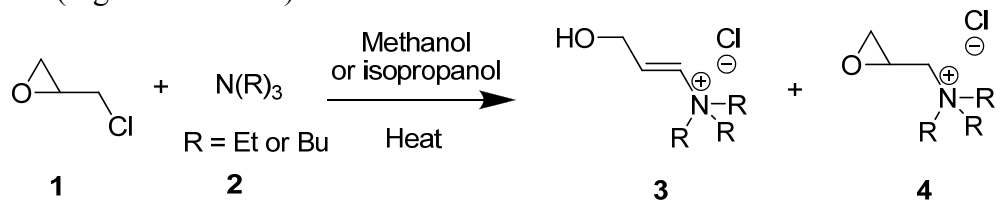


Figure 32. The preparation of glycidyltrialkylammonium chlorides.

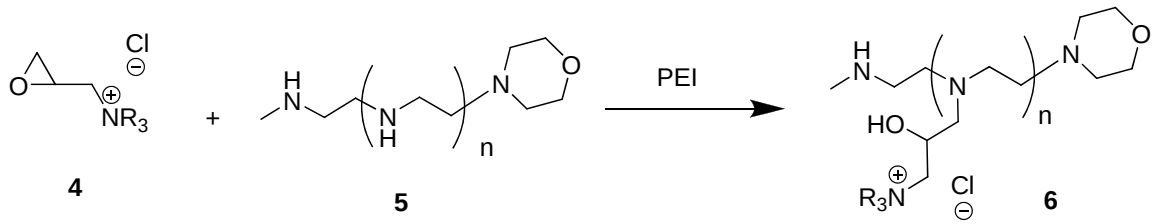


Figure 33. The reaction of glycidyltrialkylammonium chlorides with PEI.

To graft the trimethyl ammonium binding site onto the dendrigraft, the commercially available glycidyl ammonium chloride was reacted with the desired backbone in methanol under reflux (Fig. 34). The product showed well resolved NMR signals indicating a uniform product, an assumption supported by the low polydispersity factor of M_z/M_w= 1.14.

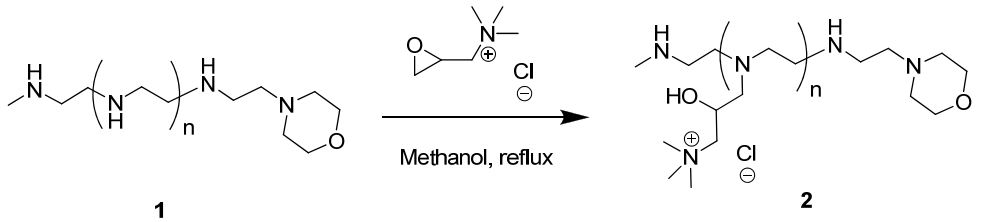


Figure 34. Synthesis of dendrigraft with trimethyl ammonium binding sites.

This method can be used for the synthesized binding sites as well; another option was to react the backbone with epichlorohydrin and then the respective trialkylamine. Both methods were attempted and it was determined that the grafting of the synthesized binding sites to the backbone resulted in a higher grafting yield. Therefore, this method has been used to synthesize all dendrigrafts with binding sites (Fig. 35).

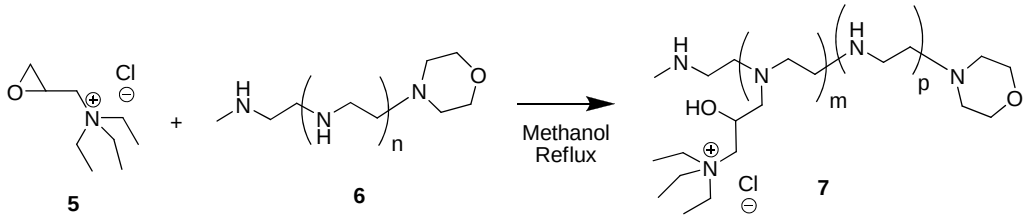


Figure 35. Synthesis of dendrigraft with triethyl ammonium binding sites.

A grid of each of the four backbones with each of the three binding sites has been synthesized. The grafting yield was reduced when with the size of the binding site or the backbone, but in all cases the grafting yield is above 70%, in most cases above 90%. The perchlorate remediation efficiency of all 12 samples was compared. This data was used for the comparison of the binding strength and regeneration efficiency depending not only on the binding site, but also the steric hindrance and capacity of the different length backbones and amounts of branching.

Initial screening was carried out to graft the three binding site onto PEI molecules and two dendrigraft polymers, G1(100-30) and G2(100-30-15). The results are summarized below. Glycidyl trialkylammonium chlorides were freshly prepared as described above by refluxing epichlorohydrin with the respective trialkylamine in methanol. After high vacuum evaporation of excess epichlorohydrin and trialkylamine, the residue was directly used without further purification. The reactions of glycidyl trialkylammonium chloride with linear PEI chain have been carried out in refluxing methanol for 16 hours. After solvent removal, the alkylated PEI were dissolved in water and purified by ultrafiltration. NMR spectroscopy was employed as the primary tool for product characterization. The grafting yield was calculated for several products based on the integration of NMR peaks. Grafting yield was calculated based on the following formula:

$G\% = 400 / 11(\text{Int } (C\underline{H_2}\text{-NH})/ \text{Int } (C\underline{H_2}\text{-N+}) - 0.182)$ (For methyl)

$G\% = 50 / (\text{Int } (C\underline{H_2}\text{-NH})/ \text{Int } (C\underline{H_2}\text{-N+}) - 0.25)$ (For ethyl and butyl)

Results are summarized in the table below.

Table1. Dendrigraft grafting comparison

Entry	PEI (equivalent of –NH)	Glycidyltrialkyl ammonium chloride	Amount (Equivalent relative to –NH)	Grafting Yield (%)
1	PEI-100	TMAC	1.1	94
2	PEI-50	TEAC	1	76
3	PEI-50	TBAC	1	73
4	PEI-100	TBAC	1	69
5	G1(100-30)	TEAC	1	74
6	G1(100-30)	TEAC	0.5	39
7	G1(100-30)	TBAC	1	52
8	G1(100-30)	TBAC	0.5	34
9	G2(100-30-15)	TMAC	1	88
10	G2(100-30-15)	TEAC	1	79
11	G2(100-30-15)	TBAC	1	76

The grafting efficiency is largely dependent on steric effects. As the size of PEI increases, the grafting efficiency decreases. For the same PEI polymer, the grafting efficiency was observed to be TMAC > TEAC > TBAC. Nevertheless, all glycidyl trialkylammonium chlorides react fairly well with dendrigraft polymers to give cationic dendrigrafts for perchlorate binding.

Moreover, a grid of all of these 12 dendrigrafts has been synthesized that are also crosslinked with PEG (550). PEG in these dendrigrafts serves three functions: make the soluble perchlorate binding-site grafted resin insoluble, strengthen the resin so that it can be used under the usual

pressure of ion exchange remediation, and reduce biofouling. The crosslinking reaction is performed by reacting the perchlorate binding site-grafted resin with commercially available PEG-diepoxide in isopropanol at 80 °C (Fig. 36).

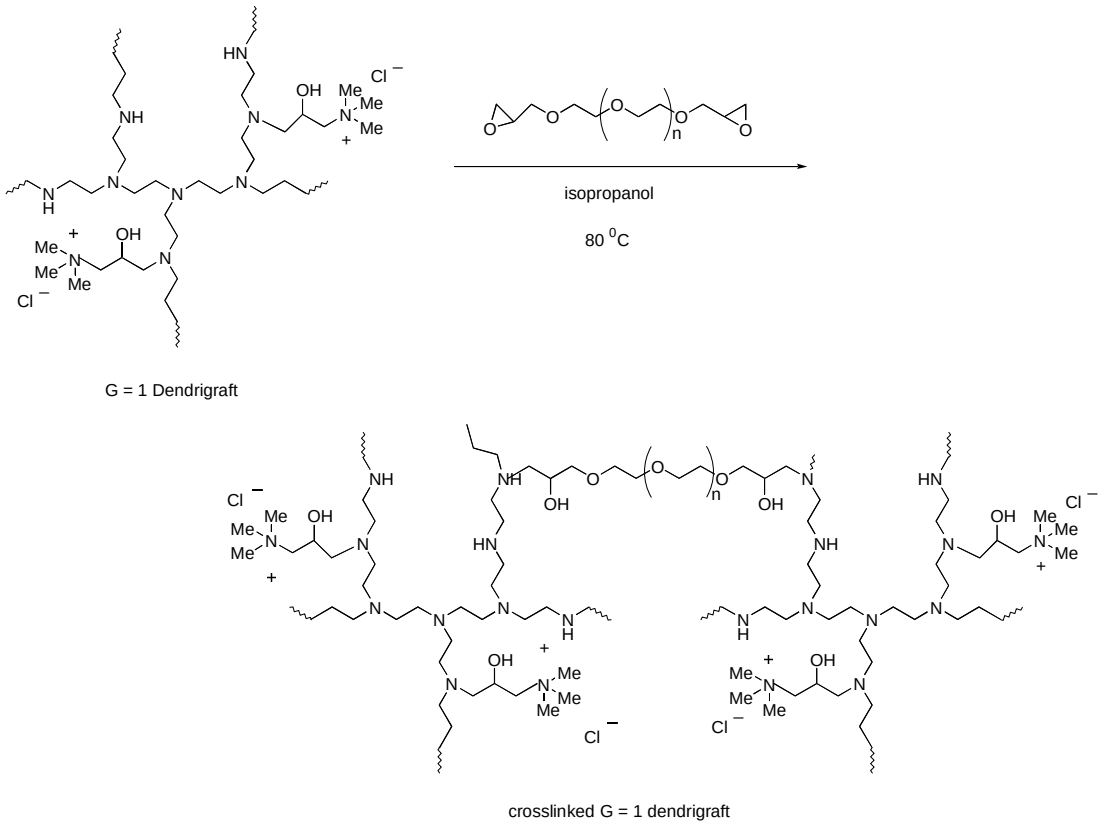


Figure 36. Crosslinking Reaction of the Binding-Site Grafted Resin with PEG.

Purification of all crude dendrigraft polymers was performed by ultrafiltration (UF) using 1, 10, and 30 kDa cut-off size exclusion membranes.

Crosslinking G1(100-30) glycidyl trimethyl ammonium chloride: The crosslinking experiments were begun using G = 1 functionalized with glycidyl trimethyl ammonium chloride and poly(ethylene glycol) diglycidyl ether as a crosslinker. Since the dendrigraft was partially functionalized with trimethyl ammonium chloride groups, there were plenty of NH groups to crosslink. The dendrigraft can be grafted with the desired level of trialkyl ammonium chloride then crosslinked. The monomer was characterized before oligomerization to give material with a known amount of exchange sites.

An alternative way of reacting the crosslinked dendrigraft with glycidyl trialkyl ammonium chloride would be problematic. The heterogeneous material would be more difficult to consistently functionalize with the glycidyl trialkyl ammonium chloride. Also, analysis of this material would be more difficult with mass balance as the only quantitative method and an infrared spectrum for qualitative information only.

The trail of experiments to find an acceptable material derived from the functionalized G1 dendrigraft and poly(ethylene glycol) diglycidyl ether totaled about sixty. The variables examined were temperature, solvent, concentration of the dendrigraft and concentration of crosslinker. The G1(100-30) dendrigraft was functionalized with 30 % glycidyl trimethyl ammonium chloride. The poly(ethylene glycol) diglycidyl ether was chosen because it should be long enough (DP = 150) to link the dendrigrafts. Shorter chains would have a greater tendency to react intramolecularly.

The temperatures examined in MeOH were 40 ^{0}C and 65 ^{0}C. These reactions produced gels after very long time frames (2 -3 days) and after very high crosslinker loading. In retrospect, the concentrations were too low. The crosslinked products from all these reactions were gels that absorbed a large amount of water (not quantified).

The reactions were then run in isopropanol to achieve a higher temperature. After several experiments with increasing the dendrigraft concentration and crosslinker concentration (based on weight %) a recipe was developed that gave a product that would swell in water and settle to the bottom of the vessel it was placed in. This allowed for a material that would not plug a column and allow adequate flow for binding and resin capacity studies. This recipe was 15 weight % crosslinker, 15 weight % solution of the dendrigraft in isopropanol and a temperature of 80 ^{0}C all with mechanical stirring.

Synthesis of glycidyl triethyl ammonium chloride, GTEAC: The reaction was initially run using 1.0 equivalent epichlorohydrin per triethylamine at 55 ^{0}C in MeOH for 2.5 hours. The isolated yield was 75 % with only a small amount of impurities (< 4 % by ^{1}H NMR). The reaction was run using 1.5 equivalent epichlorohydrin per triethylamine at 55 ^{0}C in MeOH for 2.5 hours. The isolated yield was 98 % with a small solvent impurity by ^{1}H NMR spectroscopy.

Synthesis of glycidyl tributyl ammonium chloride, GTBAC: The reaction was initially run using 1.0 equivalent epichlorohydrin per tributylamine at 55^{0}C in MeOH for 2.5 hours. The isolated yield was 47 % with some impurities (< 8 % by ^{1}H NMR). By changing reaction time to 3.5 hours isolated yield was 54 % with some impurities (< 8 % by ^{1}H NMR). When reaction was run using 1.5 equivalents epichlorohydrin per tributylamine at 55^{0}C in MeOH for 5 hours, the isolated yield was 66% with the complete removal of impurities by NMR spectra. The reaction was run neat at 55^{0}C for 5 hours to give an isolated yield of 22 %. One more reaction was run with 2.5 equivalents epichlorohydrin per amine at 55^{0}C for 5 hours in MeOH to give a pure product in an isolated yield of 97 %.

Synthesis of G1(100-30)-TMAC: TMAC was used in 40 % per NH of G = 1 PEI (100, 30) in refluxing MeOH for 24 hours. After UF (3K) in water, a yield of grafted product came to 33 % by mass balance.

Synthesis of G1(200-60)-TMAC: TMAC was used in 40 % per NH of G = 1 PEI (200, 60) in refluxing MeOH for 24 hours. After UF (3K) in water, a yield of grafted product came to 23 % by mass balance.

Synthesis of G2(100-30-15)-TMAC: TMAC was used in 40 % per NH of G = 1 PEI (200, 60) in refluxing MeOH for 24 hours. After UF (3K) in water, a yield of grafted product came to 33 % by mass balance.

Synthesis of G1(100-30)-TEAC: TEAC was used in 50 % per NH of G = 1 PEI (100, 30) in refluxing MeOH for 16 hours. After UF (3K) in water, a yield of grafted product came to 27 % by mass balance

Synthesis of G1(100-30)-TBAC: TBAC was used in 40 % per NH of G = 1 PEI (100, 30) in refluxing MeOH for 16 hours. After UF (3K) in water, a yield of grafted product came to 4 % by mass balance. Changing reaction time to 30 hours and using isopropanol (instead of methanol) did not help to increase the grafting yield.

Characterization of the Physicochemical Properties of Perchlorate Binding Priostar® Dendritic Polymers: The molecular weight of all 12 different dendrigrafts with binding sites was measured by size-exclusion chromatography (SEC) with dendrigraft standards. The molecular weight distributions were all narrow, below 1.5. The molecular weight of the crosslinked samples cannot be determined by SEC, because these samples are not soluble anymore. The size and molecular mechanical properties were studied by atomic force microscopy (AFM).

Summary:

1. The three binding sites were prepared to evaluate: 1) Grafting efficiency of binding sites with methyl, ethyl, and butyl groups onto the different dendrigraft polymers depending on the size of these sites; 2) Binding efficiency for perchlorate of these sites and complex stability; and 3) Release kinetics of perchlorate from these sites during recovery of the polymers.
2. Trimethyl, triethyl, and tributyl ammonium sites grafted onto the dendrigraft polymers were used: the triethyl and trimethyl ammonium sites are expected to bind not as strongly and thus should be easier to regenerate. The capacity in the dendrigrafts will not be increased via the strength of the binding site but via the larger number. The three different binding sites (methyl, ethyl, and butyl) are also being evaluated because of their size differences – a larger size could result in a lower grafting density due to steric hindrance, i.e., a larger binding site might not be able to penetrate the dendrigraft polymer completely. Originally these three binding sites were designed as "clickable moieties", i.e. they were going to be attached to the backbone via azide reactions. An effective method avoiding azides was found.
3. Glycidyl trimethylammonium chloride is a widely used cationic agent for many applications and commercially available at an affordable price. Glycidyl triethylammonium chloride and glycidyl tributylammonium chloride were synthesized by reacting epichlorohydrin with triethylamine and tributylamine, respectively, at room temperature for 4 days. The mixture separated into two phases. The lower phase was separated and dried by high vacuum to give the product as a semi-crystalline residue.
4. All binding sites were characterized by 1H NMR and IR spectroscopy to confirm their structure and purity.
5. Developed a synthesis to prepare crosslinked dendrigrafts using poly(ethylene glycol) diglycidyl ether.

6. Improved the preparations of triethyl glycidyl ammonium chloride and tributyl glycidyl ammonium chloride for use in preparation of dendrigrafts functionalized with trialkyl ammonium chloride.
7. Examined the reactions of the three trialkyl glycidyl ammonium chloride with the dendrigrafts for yield, reaction time and workup.
8. General conclusions about the relative reactivity of the glycidyl trialkyl ammonium chlorides with G =1 and 2 PEI dendrigrafts: Trimethyl > triethyl >> tributyl under the same conditions.
9. Grafting yields have been obtained thus far in the range of 15 – 45 % of NH for trimethyl and triethyl

3. Scale-Up Synthesis and Purification

Three main approaches have been found to optimize synthesis and purification steps: 1) Reaction condition optimization; 2) click chemistry; and 3) crosslink without removing excess PEI by ultrafiltration.

Reaction condition optimization:
Based on this initial screening, dendrigraft polymer G1(100-30) was selected for scale-up to produce a 100-g batch of purified product. This material was needed for the development of performance tests, including tests of polymer porosity/pore size and water flow rates. The reaction was performed in boiling toluene. After complete reaction the mixture was cooled to room temperature, and the clear toluene layer separated from precipitated material. Toluene was removed under vacuum to give 45 g PEOX DP= 30 from a total crude product yield of 216 g. This PEOX contained only a trace of dendrigraft 100/30 G= 1 as determined by SEC. The precipitate was found to contain a mixture of product dendrigraft 100/30 G= 1 and excess PEOX DP= 30, and was purified by UF through a 30 kDa cut-off membrane.

In the small scale dendrigraft synthesis, a four time excess of oxazoline to free amines in the backbone was used to increase the grafting density. The excess oxazoline (and free PEOX chains) have to be removed via purification, adding an additional step and producing waste during the synthesis. Oxazoline is also the most expensive part of this synthesis. For scale-up a one-to-one ratio of oxazoline to free amine would be preferred, if not less. The excess has been reduced from four times to two times, which resulted in a decrease of grafting density of only two percent. It was also found that hot, instead of cold, toluene was more effective for the extraction of excess PEOX, reducing the duration (number of circulations) of the UF purification.

Reduction of amount of reagents:
The most costly step in the preparation was determined to be the grafting of PEOX onto the PEI core or structure. In the small scale process a living end PEOX was reacted with 50% excess per NH of PEI or 0.5 equivalents per NH. The grafting yield was typically 20 % or 20 NH of 100 available NH grafted with PEOX with the unreacted PEOX removed by ultrafiltration in the lab or on scale-up some precipitation method. Initial experiments that reduced the PEOX from 50 % to 40 % per NH of the PEI core gave a 15 % grafting and a reduction of the PEOX from 40 % to

30 % per NH of the PEI core gave a 10 % grafting. An alternative strategy was examined using click chemistry or the Huisgan reaction. This is a cyclo-addition reaction of an alkyl azide and acetylene to form a triazole. In this synthetic design the PEOX was initiated with propargyl tosylate and the core was functionalized with an azide by a reiterative addition of epichlorohydrin then sodium azide. In principle the click reaction should be capable of a complete reaction of PEOX onto the core.

Scale up Reaction Optimization with Click Chemistry: Scale up of G1(200-60) Dendrigraft Synthesis using Click Reaction:

Objective was to develop a synthesis that grafts all the propargyl initiated PEOX onto the azide functionalized PEI or PEOX – PEI copolymer. Successful execution of this synthetic plan eliminates a costly purification step and converts all starting material into product. The procedure to prepare the PEI hydroxypropylazide using a one pot procedure with epichlorohydrin – sodium azide was replaced with two readily accessible intermediates, derived from the reaction of epichlorohydrin and sodium azide in the presence of acetic acid, which can react with PEI to give any degree of substitution desired (Figure 37). The click reactions of propargyl-initiated PEOX with PEOX–PEI and PEI hydroxypropylazide derivatized with this new procedure were examined with copper catalyst and under thermal conditions.

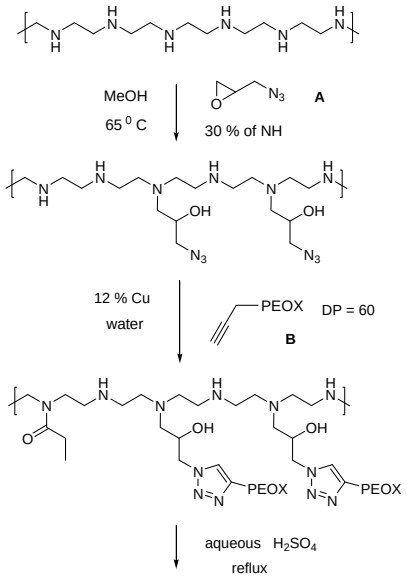


Figure 37. Preparation of G1(200-600) via click chemistry.

The most costly step in a dendrigraft preparation was determined to be the grafting of PEOX onto the PEI core or structure. It is this step that is being addressed, since the other steps are much less costly. Several click reactions were found to work, however, the yields were very low, ~ 5%, and were all consistently the same. Although the azide peak in the IR spectrum was strong, indicating a relatively high azide loading, the other signals for the hydroxypropyl azide in the ^{13}C NMR were very broad, as would be expected for a polymer NMR spectrum. It was thus decided to prepare the hydroxypropylazide by a different method. Using the same epichlorohydrin – sodium azide combination, two intermediates, an azidoepoxide and chlorohydroxypropylazide, were prepared and characterized. These were then reacted with the PEI polymer to give the PEI – hydroxypropylazide. Using the PEI hydroxypropylazide prepared by this new procedure the click reaction with propargyl initiated PEOX was examined.

Crosslink dendrigrafts without removing excess PEI by ultrafiltration:

The solution of living end PEOX (DP60, 25.55 g, 0.2578 mmoles) was added into PEI (DP200, 0.46 g, 10.69 mmoles) to form dendrigraft with some free PEOX. Free PEOX was not removed through ultra filtration. This mixture was hydrolyzed to form PEI. Four grams of this PEI sample was refluxed with Glycidyl TMAC (6.32 g) to yield 9.73 g of G1(200, 60)-TMAC. Five grams of

this G1(200-60)-TMAC sample was cross-linked using following conditions: cross-linker: 1.5 g, 30%; isopropanol: 56.6 g, temperature: 80 ^{o}C; time: 5 hours. Total capacity test was carried out with this sample to know its binding capacity for perchlorate, nitrate and sulfate.

Summary:

1. In the living end PEOX grafting the most costly step in the preparation was determined to be the grafting of PEOX onto the PEI core or structure. Free PEOX was removed by UF and hence adding cost to the synthetic method. Approximately 20% PEOX grafting was attained. Reduction of stoichiometry further reduces the grafting efficiency.
2. Click chemistry approach showed promise but produced low yield (5 %)
3. Crosslink dendrigrafts without removing excess PEI by ultrafiltration approach worked the best and very cost-effective

4. Materials' Stability and Physicochemical Properties

Hydrolytic Stability of PriostarTM Dendritic Polymers with Perchlorate Binding Sites:
For hydrolytic stability we have anecdotal data during synthesis that polymer is stable from pH 2-13: During the synthesis the pH ranges from pH 2 to pH 13, and no break-up products have been observed during the synthesis.

Porosity by Porosimeter and Atomic Force Microscopy:
Crosslinking the resin results in a mechanically stronger material, but because a network of polymer chains is formed it also changes the pore size of the resin. The pore size, though, is important for perchlorate binding because access to binding sites and rate of binding might be changed in the process.

We measured the pore size by a porosimeter; in that method the sample is evacuated and known amounts of gas are pulsed through the sample. This size does not accurately describe the pore size in water, since it has already been seen that the resin swells in water.

Porosity data
To determine the porosity of all backbones the PET surface area and pore size was determined with an ASAP2020 porosimeter (Table 2).

Table 2. BET surface area and pore size for soluble dendrigrafts with binding sites.

Sample	BET surface area (m^2/g)	Pore Size (Å)	Sample	BET surface area (m^2/g)	Pore Size (Å)
G1(100-30)-TMAC	0.158	32.9	G2(100-30-15)-TMAC	0.104	33.3
G1(200-60)-TMAC	0.057	N/A	G2(200-60-30)-TMAC	0.073	100.2
G1(100-30)-TEAC	0.002	101.6	G2(100-30-15)-TEAC	N/A	2655
G1(200-60)-	0.052	24.1	G2(200-60-30)-	0.011	N/A

TEAC			TEAC		
G1(100-30)-TBAC	0.042	58.8	G2(100-30-15)-TBAC	0.040	N/A
G1(200-60)-TBAC	0.139	44.5	G2(200-60-30)-TBAC	0.126	25.3

Porosity measurements work by removing all gas via a strong vacuum and ten pulsing in known amounts of nitrogen gas. The pressure of nitrogen gas passed the sample is measured. With that information surface area and pore size of the sample can be calculated y using different theories. In this research the BET theory is employed.

The non-crosslinked samples are very soft; in this case these porosity measurements result in erroneously low surface area and pore size values, because the pore walls collapse during application of the vacuum. On the other hand, the comparison of these values can still show trends. Initial data suggests that a backbone with longer side chains results in higher surface area and pore size.

To determine pore sizes and particle sizes more accurately, we performed Atomic Force Microscopy on the G1(200-60)-TMAC resin (Fig. 38). The data was then analyzed for particles size and pore size. As expected with crosslinked samples the particles are not made from single polymer chains but polymer chain aggregates. Grain distribution analysis shows that most of the grains are 500 -750 nm in diameter (Fig. 39), and the pores are 400 – 800 nm in diameter (Fig. 40).

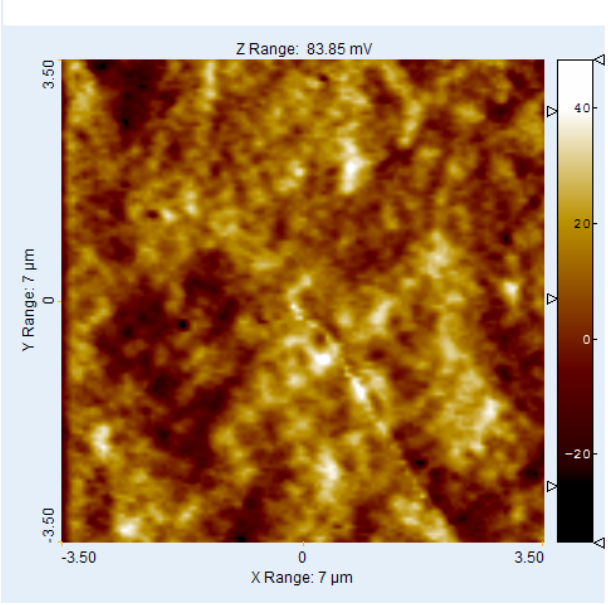


Figure 38. Topography of a G1(200-60)-TMAC film, measured by AFM.

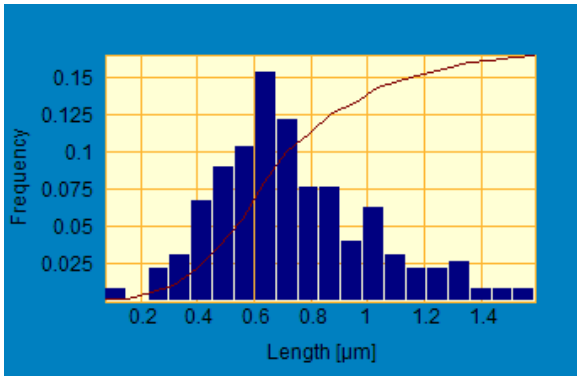


Figure 39. Grain size analysis of G1(200-60)-TMAC resin film.

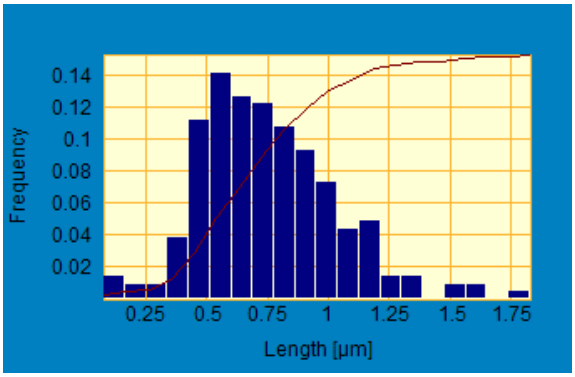


Figure 40. Pore size analysis of G1(200-60)-TMAC resin film.

The small grain size allows or a high surface area, increasing the access of perchlorate to the binding sites. The pores themselves are also large enough for perchlorate to pass through freely. Thus the structure of the resin increases the efficiency of the resin

Measure Biological Stability and Toxicity of PriostarTM Dendritic Polymers with Perchlorate Binding Sites:

The cytotoxic effects of the dendrigrafts synthesized were investigated in Caco-2 (human colon epithelial) cells using MTT assays. The MTT (Acros Organics) assay is a colorimetric assay in which a yellow tetrazolium salt (MTT) is reduced to purple formazan crystals by metabolically active cells. The crystals are then solubilized and color intensity quantitated spectrophotometrically. This assay yields an IC_{50} value; the concentration at which the mitochondrial function of 50% of cells is inhibited. The lower the IC_{50} value, the more toxic the substance being tested.

Nine dendrigraft samples synthesized and were tested in the Caco-2 cytotoxicity studies. Results from the experiments are shown in Table 3. For the most part, the IC_{50}'s of the dendrigraft samples decreased with an increase in terminal group carbon chain length; methyl ≈ ethyl < glycidyl tributyl ammonium chloride samples. The resulting toxicity patterns could be due to an increase in molecule flexibility via the increase in carbon chain length, thus facilitating detrimental interactions with cell membranes. Alternatively, the larger dendrigraft (TBAC) might retain small impurity molecules from the synthetic process which dissociate in cell culture conditions and in themselves have a negative effect on cell viability.

Table 3. Cytotoxicity measured as IC_{50} values of soluble dendrigrafts in Caco-2 cells via an MTT assay.

Sample	50% Viability (ug/well)	IC_{50} (mg/mL)
G1 (200,60)- TMAC	1.3	0.013
G2 (100,30,15)- TMAC	4.5	0.045
G2 (200,60,30)- TMAC	1.25	0.013
G1 (200,60)- TEAC	2.06	0.021
G2 (100,30,15)- TEAC	2.9	0.029

G2 (200,60,30)- TEAC	3.5	0.035
G1 (200,60)- TBAC	0.62	0.006
G2 (100,30,15)- TBAC	1.06	0.011
G2 (200,60,30)- TBAC	0.44	0.004

5. Total Capacity

Soluble Dendrigrafts

The dendrigraft backbones are water soluble, as well as the un-crosslinked backbones with added binding sites. Thus, an ultrafiltration (UF) method was developed to determine perchlorate binding instead of the commonly-used total capacity measurements of solid resins (Fig. 41). The

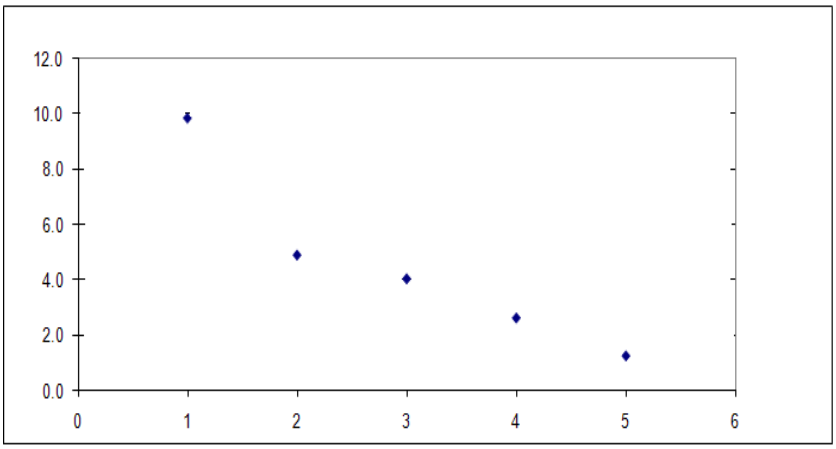


Figure 41. G1(100-30)-TMAC with 50ppm of perchlorate, 4 recirculations of ultrafiltration, measuring perchlorate concentration in permeate.

dendrigraft with binding sites is combined with $NaClO_4$ (50 ml, 0.20M) and the resulting solution was stirred for one hour. The solution was then purified by ultrafiltration with a 10 kDa regenerated cellulose membrane, 4 recirculations, collecting 100 ml of permeate. A sample of retentate was collected after each recirculation. The perchlorate concentration of both permeates and retentates were measured by ion electrodes. Dendrigrafts were tested with high perchlorate concentrations to ensure that even in the presence of high nitrate and sulfate concentrations the capacity for perchlorate is still high. With large capacities the resin is expected to be effective for the removal of low or high concentrations of perchlorate.

Choice of Binding Sites:

To determine the best binding sites the G1(100-30) backbone was substituted with three possible binding sites: trimethyl ammonium chloride (TMAC), triethyl ammonium chloride (TEAC), and tributyl ammonium chloride (TBAC). TBAC is commonly used with a hydrophobic resin to result in a strong-base resin. These are usually difficult to regenerate. In this project trimethyl- and triethyl-bindng sites were tested as well. It was expected that these smaller alkyl groups result in weaker binding and possibly less selectivity, but possible regeneration. The goal is to have enough of an increase in capacity that even though the selectivity is reduced, still all perchlorate will be removed.

A solution of 1 ppm, 10 ppm, 100 ppm, 1,000 ppm and 10,000ppm sodium perchlorate-dendrigraft (G1(30-100)-TMAC, G1(30-100)-TEAC and G1(30-100)-TBAC solutions were made and filtered through ultrafiltration membranes. Since the limit of detection of the perchlorate ISE is around 1 ppm, the results for the 1 ppm perchlorate exposure study have a

high degree of error. For the 10ppm and 100ppm studies, more that 99% of the perchlorate was removed for all three functional groups after two or more recirculations.

Permeate **Retentate**

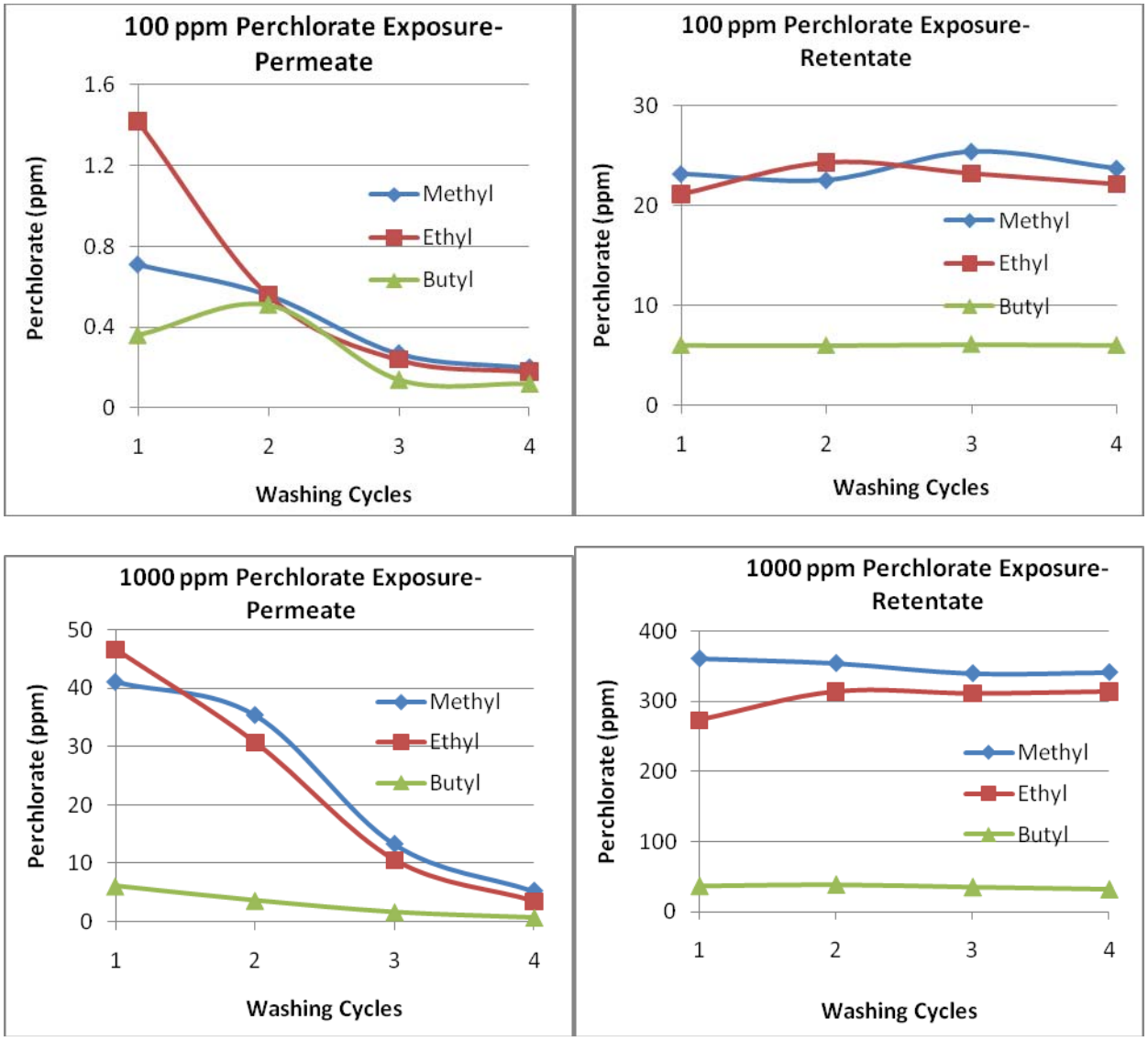


Figure 42. Comparison of perchlorate retention of dendrigrafts with a G1(100-30) backbone with TMAC, TEAC, and TBAC binding sites.

For 1,000 ppm and 10,000 ppm load, no major differences were found between the TMAC and TEAC binding site dendrigrafts, which showed binding efficiencies of about 98.5% and 90% removal of perchlorate. However the butyl samples were shown to have more efficient binding for perchlorate at 1,000 ppm load with 99.8% removal of perchlorate after two or more recirculations.

All three dendrigrafts gave clear solutions except G1(100-30)-TBAC dendrigraft. G1(100-30)-TBAC dendrigraft with 1,000 ppm sodium perchlorate gave cloudy/milky solution but it was good enough for ultrafiltration studies. But samples collected from G1(100-30)-TBAC dendrigraft retentate were turbid where as permeates were clear from same experiment. On the other hand, 10,000 ppm sodium perchlorate-G1(100-30)-TBAC dendrigraft solution was unsuitable for ultrafiltration due to precipitation. But, this indicates that at high perchlorate exposures, the dendrigraft captures the perchlorate molecule and renders the final product hydrophobic or creates a cross-linking effect.

A total of 112 samples were collected and tested for perchlorate concentration (see Figure 42). These data demonstrate:

1) For the 100 ppm through 1000 ppm experiments, the retentates for the methyl and ethyl dendrigrafts retained and release similar perchlorate concentrations. At 1000 ppm butyl was better than methyl and ethyl. With higher exposure levels, the retentates showed a proportionate amount of perchlorate released.
2) The butyl samples show that the dendrigraft binds strongly to perchlorate and decreases its leakage into the extraction solvent which demonstrates tighter binding characteristics than either methyl or ethyl functional groups.
3) Up to 100 ppm perchlorate concentrations, each functional group performs similarly after two recirculations. The tributyl ammonium functional group works well after just one recirculation.
4) The trimethyl and triethyl ammonium functional groups dendrigrafts are at a greater risk of leaching once the material becomes saturated.
5) After 1000 ppm methyl and ethyl start losing their perchlorate binding capability but butyl remained the same as for 100 ppm

Since the goal of this project was to develop a regenerable resin, the TBAC binding site was excluded at that point. Also, at high concentrations of perchlorate the resin precipitated. As much as crosslinked, solid resins were eventually used, this still indicates that the behavior of the TBAC resin would change considerably, e.g. the density would increase, likely resulting in increased pressure of operation.

Not much of a difference was seen between the TMAC and TEAC binding site. Since the synthesis of the TMAC binding site is less expensive, TMAC-substituted dendrigrafts were used for future development.

The data also clearly shows that we have the option, if needed, to develop a strong-base perchlorate-binding resin by using the TBAC dendrigraft resins.

Choice of Backbone:

To determine if the size and amount of branching affects perchlorate binding, G1(100-30), G1(200-60), G2(100-30-15), and G2(200-60-30) were substituted with TMAC binding sites, and capacity was again determined by the ultrafiltration method for soluble resins (Figure 43). It was shown that both G1(200-60) and G2(100-30-15) were the most effective in removing

perchlorate. Since a G1 dendrigraft is cheaper to synthesize, G1(200-60)-TMAC became the lead candidate.

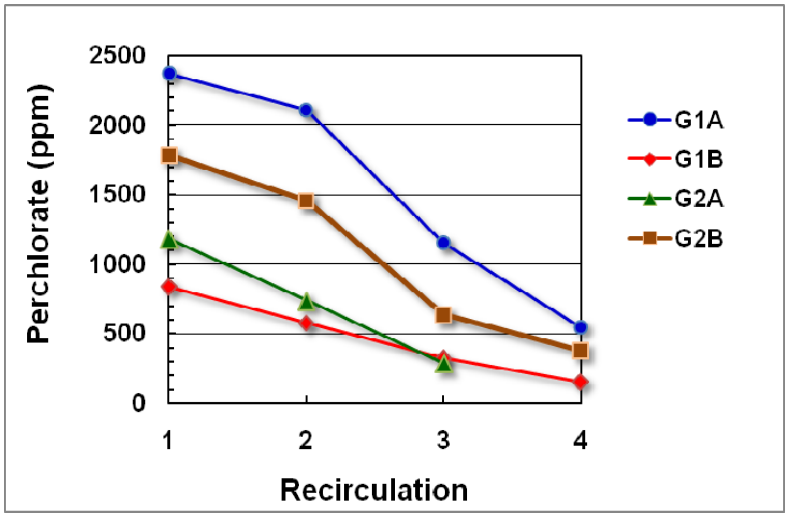


Figure 43. Graph comparing the perchlorate concentration as a function of time in a spiked water sample. The original perchlorate concentration was 10,000 ppm. Each dendrigraft was functionalized with TMAC ligands for extraction of perchlorate.

Total Capacity of soluble G1(200-60)-TMAC:

To calculate a total binding capacity or milli-equivalent (meq) binding capacity for the lead candidate, a fixed amount of perchlorate was exposed to varying quantities of dendrigraft in order to reach a saturation level. In four experiments with varying exposure levels, all four runs showed that after four recirculations using a cross-flow apparatus, the dendrigraft material absorbed about certain amount of a 100 mL - 10,000 ppm perchlorate solution. We measured the amount of perchlorate in permeate, retentate solution, and calculated the total amount of perchlorate absorbed by the dendrigraft. The data in Table 4 shows the amount of perchlorate that was absorbed relative to the amount of dendrigraft materials. With longer exposure times (i.e. further recirculations), the amount of absorbed perchlorate could further increase, but these experiments demonstrate that in homogenous mode (non-crosslinked), these materials exhibit extremely high capacities to absorb perchlorate ions.

Table 4. Binding capacity values for G1(200-60)-TMAC as a function of dendrigraft material exposed to a 10,000 ppm solution of perchlorate. Final perchlorate quantities determined from the fourth recirculation (or longest exposure time) between perchlorate and the dendrigraft material

Absorbed Perchlorate (mg)	Dendrigraft Mass (g)	Binding Capacity (meq)
811	1	8.2
480	0.5	9.7
275	0.25	11.1
245	0.125	19.8
339	0.05	68.4
323	0.01	325.8

For one gram of resin to have a 1 meq rating, it would have to absorb 99 mg of perchlorate. The data below clearly shows that using 0.01 g dendrigraft produces 325.8 meq binding capacity. The high binding capacity is extraordinary compared to the commercial resins with 1- 2 meq binding capacity. It appears that further experiments are needed to find a breakthrough point in which a high perchlorate concentration does not get absorbed. This data also suggests that there is a relationship between the amount of dendrigraft and its ability to capture perchlorate from a certain volume of effluent.

Use of ultrafiltration during dendrigraft synthesis was adding the cost to the overall process. To reduce the cost, a new method was devised to synthesize dendrigrafts without using ultrafiltration (Non-UF). Thus formed cross-linked non-UF dendrigraft G1(200-60)-TMAC was evaluated for the total binding capacity of anions, regeneration with NaCl and effect of NaCl concentration on the regeneration efficiency. As observed in Table 5, dendrigraft synthesized by non-UF approach showed significantly similar perchlorate binding capability compared to the UF approach (71.4% Vs 64.8%). Hence, non-UF approach can be used to synthesize dendrigraft in the large scale to reduce the cost of the dendrigraft product.

Table 5. Perchlorate binding of cross- linked dendrigraft G1(200-60)-TMAC prepared with or without UF in a fixed bed application

Percentage Perchlorate Binding	
Non UF	UF
71.4	64.8

Insoluble dendrigrafts:

All dendrigrafts were tested with high perchlorate concentrations to ensure that even in the presence of high nitrate and sulfate concentrations the capacity for perchlorate is still high. With large capacities the resin is expected to be effective for the removal of low or high concentrations of perchlorate. All the dendrigrafts tested showed some binding capacity for nitrate and sulfate as well as perchlorate as illustrated in Figure 44. However, G1(200-60)-TMAC exhibited the least affinity toward the nitrate and sulfate ions. It also showed some of the best ability to bind perchlorate. The cross-linked samples are showing a diminished perchlorate binding capacity relative to the homogenous or un-crosslinked samples. It should be noted that the experiments performed on the crosslinked dendrigrafts were run using an “in line” or flow through fixed bed configuration. The exposure time between resin material in these experiments is much less than with the cross-flow analysis shown above. We have clearly shown that with the homogenous experiments, the amount of time (recirculations) has a big impact on the total amount of perchlorate removed. From all the data shown here in Figure 44, G1(200-60)-TMAC is one of our lead candidates because of its ability to bind perchlorate and lack of binding towards nitrate and sulfate along with its relative ease of manufacturing. These results confirm this decision to further focus the scale-up and performance testing on materials/resins made from G1(200-60)-TMAC.

The selectivity of the different resins could be determined as well. G1(200-60) dendrigraft backbone showed some binding for chloride, perchlorate, sulfate and nitrate ions (Figure 45).

G1(200-60) bounded with 31.4% chloride on the first exposure with 1M NaCl. Non-selective binding (90-95%) of sulfate and nitrate ions was demonstrated by the dendrigraft backbone G1(200-60). The backbone also showed higher binding (72.5%) for perchlorate ions. Perchlorate removal capacity of G1(200-60) was almost similar to the G1(200-60)-TMAC dendrigraft (64.8%).

Effect of grafting TMAC on G1(200-60) backbone was evident with nitrate and sulfate ions, where G1(200-60)-TMAC showed 1% and 26.5%, respectively, binding for nitrate and sulfate ions compared to 90-95% for G1(200-60). G1(200-60) backbone is as efficient as G1(200-60)-TMAC for perchlorate removal but lacks specificity for removal of nitrate and sulfate shown by G1(200-60)-TMAC.

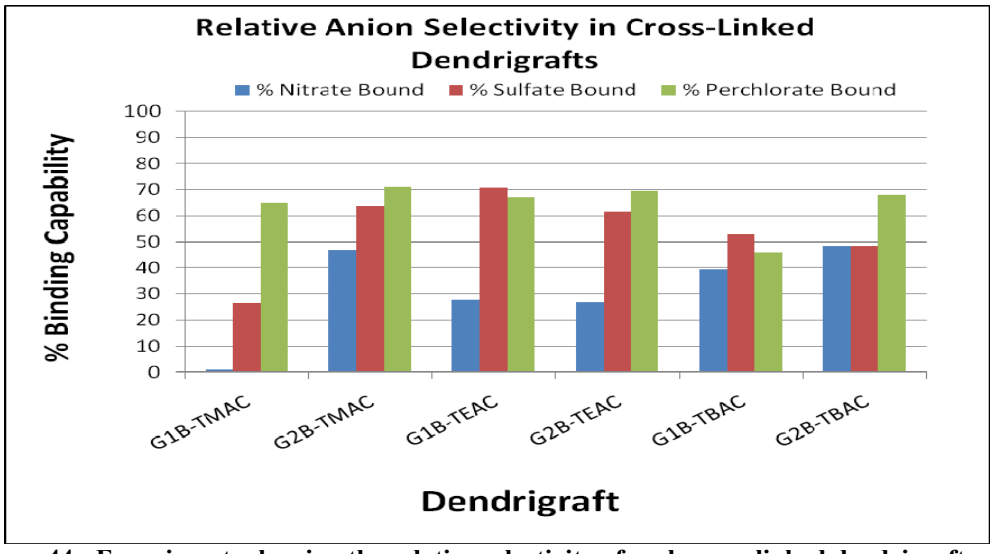


Figure 44: Experiments showing the relative selectivity of each cross-linked dendrigraft resin when exposed to nitrate, sulfate, or perchlorate ions.

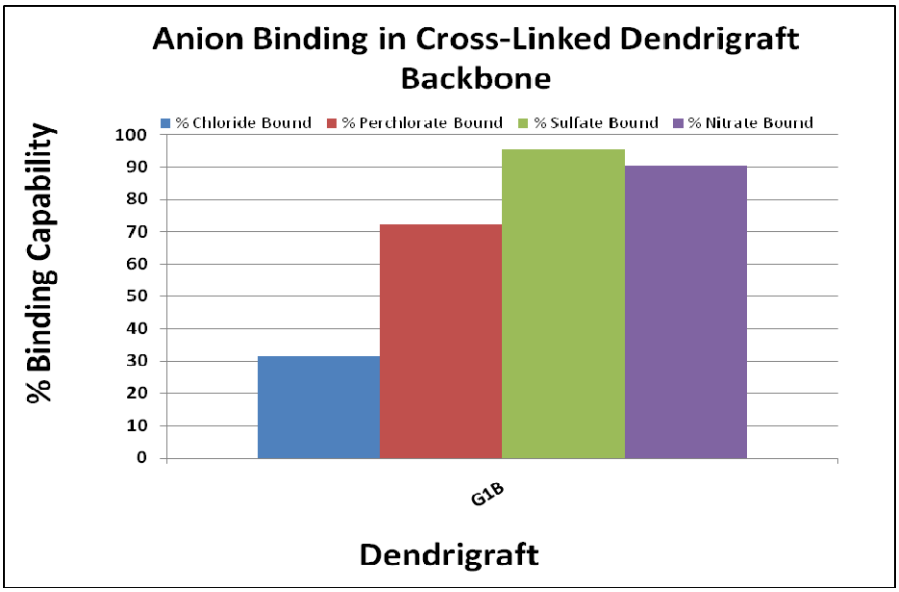


Figure 45: Experiments showing the relative selectivity of a cross-linked dendrigraft backbone resin G1(200-60), when exposed to chloride, nitrate, sulfate, or perchlorate ions.

6. Adsorption Isotherm and Kinetic Measurements

The adsorption isotherm was determined for the G1(100-30) and G2(100-30-15) backbones. The measurement was performed as a batch test with 1220 ppm perchlorate; Figure 46 shows amount perchlorate (ppm) remaining in solution over time. This high concentration was chosen to determine how much excess capacity these resins have.

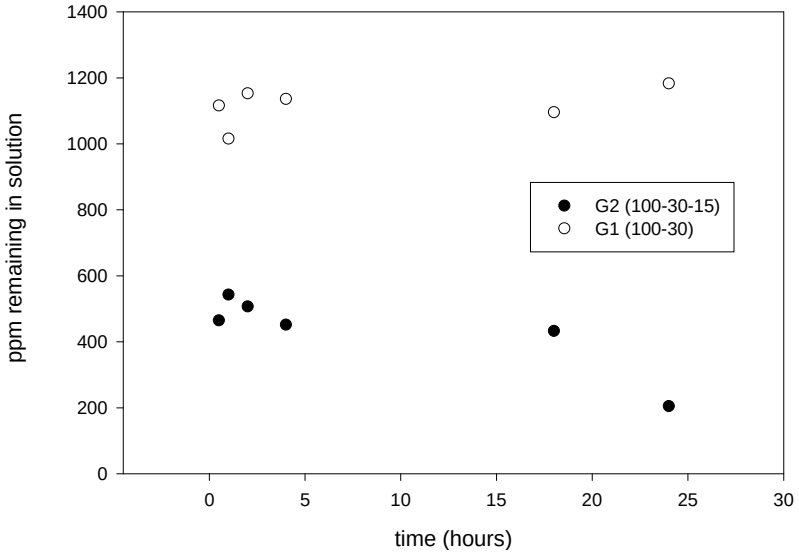


Figure 46. Adsorption isotherm of G1(100,30) and G2 (100,30,15) with 1220ppm perchlorate.

Adsorption for different concentrations at constant time (4 hrs) was also determined as a batch test for G1(100-30) and G2(100-30-15). Figure 47 shows amount perchlorate (ppm) remaining in solution after 4 hours.

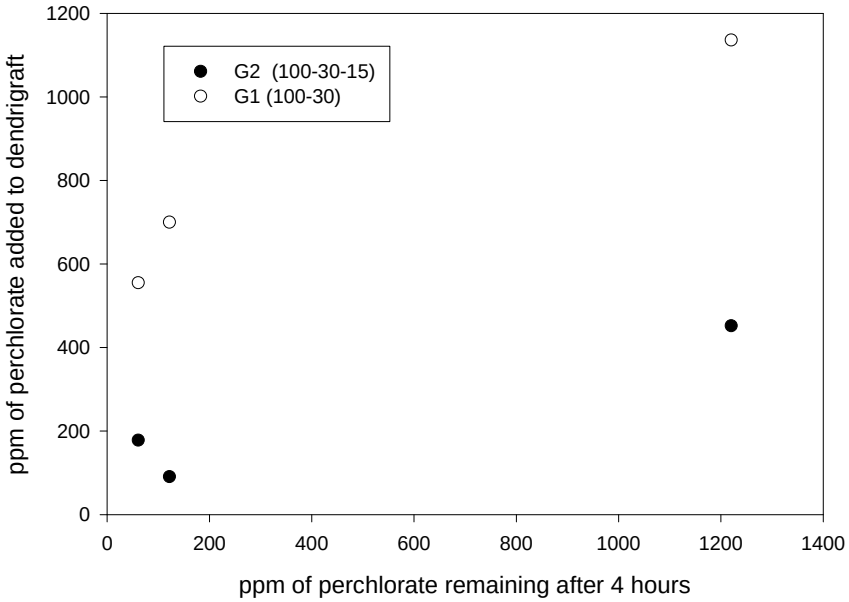


Figure 47. Adsorption at 4 hours at variable concentrations for G1(100-30) and G2(100-30-15).

Even though these dendrigrafts do not contain binding sites, G2 still binds perchlorate strongly enough to remove the majority of 12,000 ppm of perchlorate from solution.

7. Operational Properties

Initial experiments were performed to evaluate the operational capacities under pressure. It was found that the G1(200-60) crosslinked backbone withstood 5 psi of pressure, and the pressure did not rise over 24 hours.

Dendrigrafts were treated with NaCl for the determination of regenaration efficiency. Ion chromatography was used to estimate chloride content in anions treated dendrigrafts (Figure 48). Dendrigrafts treated with perchlorate showed chloride content in the range of 49-56 ppm. Dendrigrafts containing TEAC groups after being treated with either sulfate or nitrate showed significantly higher chloride content (1100-1700 ppm) compared to the TMAC and TBAC groups (74-160 ppm). This shows that dendrigrafts containing TEAC groups has slightly less perchlorate binding capability compared to the dendrigrafts containing TMAC and TBAC groups.

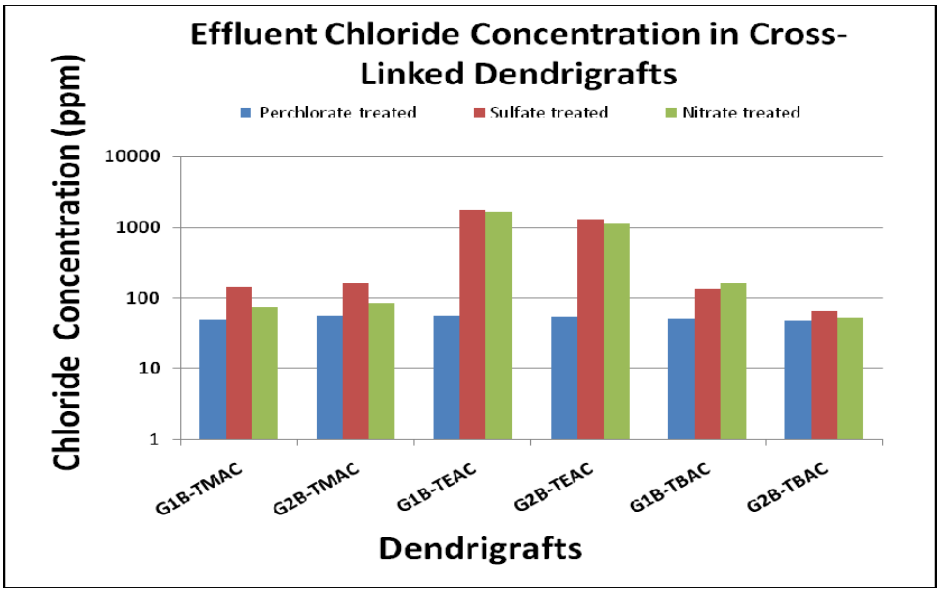


Figure 48: Graph comparing effluent chloride concentration in the cross-linked dendrigraft samples for total capacity analysis after regeneration.

Cross-linked G1(200-60)-TMAC dendrigraft was compared with its cross-linked backbone G1(200-60) for effluent chloride concentration (Figure 49). G1(200-60) showed higher chloride concentration compared to G1(200-60)-TMAC for perchlorate and sulfate treated samples, whereas no significant difference was observed for nitrate treated samples.

G1(200-60)-TMAC is showing more binding affinity towards chlorides compared to the G1(200-60), but similar binding affinity for nitrate treated samples indicate the protocol used may have produced the discrepancy in the results. Dendrigraft samples were treated with the following order: HCl, perchlorate, sulfate, and nitrate. Between each step dendrigrafts were regenerated with NaCl and then washed with DI water. Significant difference in the initital steps (before perchlorate and sulfate treatment) could be due to the difference in the equilibrium for NaCl and DI water washings with G1(200-60) and G1(200-60)-TMAC. After nitrate treatment, both the dendrigrafts were fully equilibrated and showed similar effluent chloride concentrations.

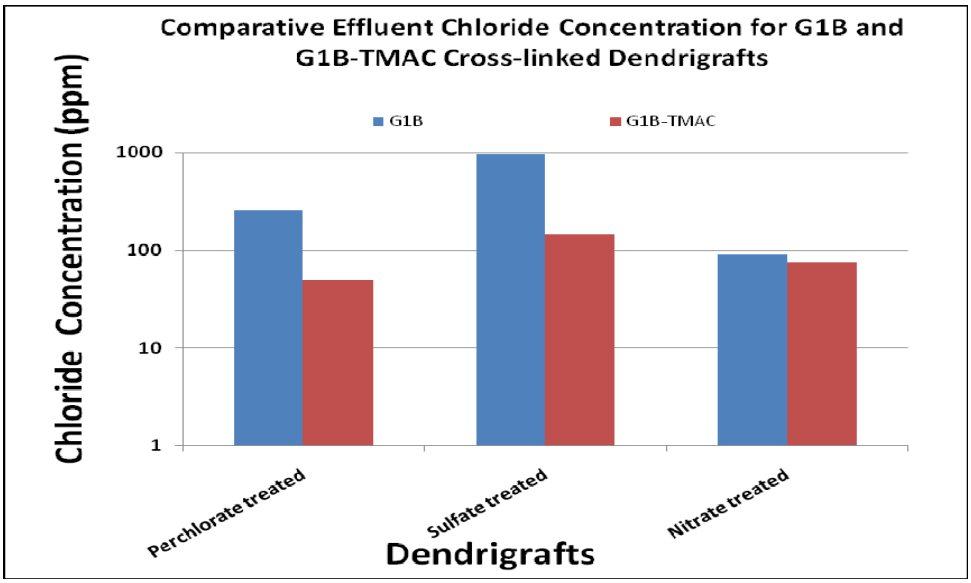


Figure 49. Comparative effluent chloride concentration for G1(200-60) and G1(200-60)-TMAC cross-linked dendrigraft samples for total capacity analysis.

To scale the synthesis of dendrigraft polymers for perchlorate removal to industrial scale, a procedure was developed that did not use the UF step for purification (see section about scale-up). The resulting resins were tested for regeneration efficiency. Non-UF dendrigraft showed similar efficiency as a regular dendrigraft to remove perchlorate from the solution. Percentage binding capacity of non-UF dendrigraft was also evaluated with perchlorate, sulfate and nitrate anions in a single experimental set-up (Figure 50). Regeneration capacity of the cross-linked dendrigraft G1(200-60)-TMAC was tested using NaCl at low concentration (0.1M instead of 1M). Total capacity protocol was used as discussed previously. Percentage binding was calculated from the source concentration after IC measurement. Anion solutions were passed through the fixed bed setup, without any dendrigraft, to identify if anions were retained within the fixed bed setup (blank solutions, data not shown).

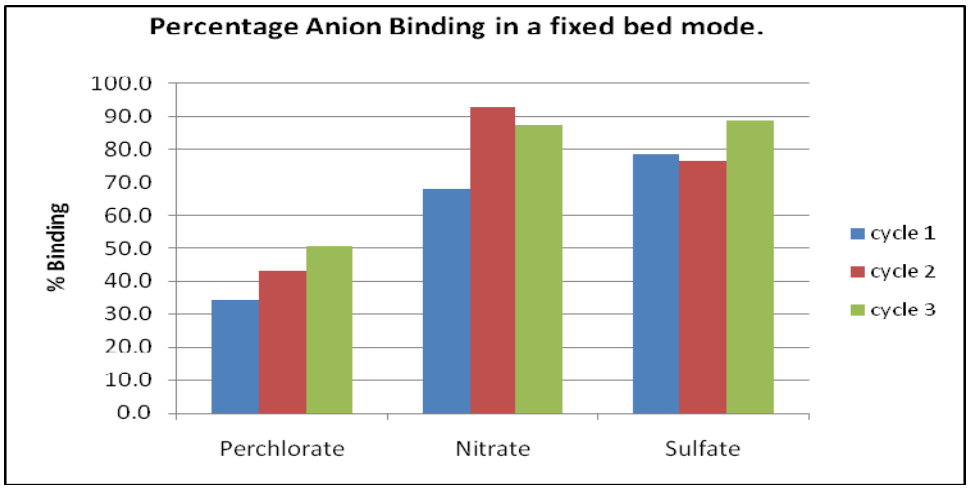


Figure 50. Percentage anion binding with cross-linked dendrigraft (200-60)-TMAC synthesized by non-UF approach.

All the resins tested showed non-specific bindings of nitrate, sulfate and perchlorate. Comparatively less binding towards perchlorate was observed (perchlorate binding: 35-50% binding). Blank anion solutions did not show any binding with the fixed bed set-up (without dendrigraft), which means anions are not sticking to the fixed bed set-up (without dendrigraft). This was done to eliminate all kind of background errors.

Dendrigraft showed significant binding to all the anions tested in this experiment. Regeneration of dendrigraft was carried out with NaCl. After regeneration anions were again passed through the dendrigraft; this cycle was repeated 3 times (cycle1, 2 and 3). Figure 50 clearly demonstrates that dendrigraft-anion complex can be regenerated with NaCl, binding increased for each cycle of regeneration.

Total Capacity for Crosslinked Strong-Base Resin

The total capacity for the strong-base G1(200-60)-TBAC (15% crosslinking) was determined. The cross linked dendrigraft was incubated with DI water for 4 h at RT and then quaternized with HCl. A fresh resin was used for all the experiments with three different anions. A total of 40 samples were collected (40 x 100 ml). In the end, column was washed with 3x100 mL of DI water (labeled as wash 1, 2 & 3). Samples 1, 10, 20, 30, and 40 were analyzed separately.

The dendrigraft showed non-specific bindings to nitrate, sulfate and perchlorate anions. Adsorption profiles of dendrigraft were not linear for all the anions tested, indicating saturation of the dendrigraft. At 1000 ml bed volume dendrigraft demonstrated maximum binding capacity (3.32 meq/g) for perchlorate (Table 6) and afterwards decreased to 2.7 meq/g at 4000 ml.

Table 6. Binding capacity of cross-linked dendrigraft G1(200-60)-TBAC with various anions in a fixed bed application with 4000 ml volume.

	Binding Capacity: Anion (meq)/Resin (g)		
Cycle (mL treated)	*Perchlorate*	*Nitrate*	*Sulfate*
cycle 1 (100 ml)	0.89	1.48	2.65
cycle 1-10 (1000 ml)	3.32	4.61	7.66

cycle 1-40 (4000ml)	2.73	2.45	7.27

At the end of the experiment dendrigrafts were washed with water and it was observed that anions were not leaking from the resin after treatment. This behavior provides higher safety profile to dendrigrafts, as dendrigraft–anion complexes are strong enough to withhold the medium strength (water) and hence will not cause any leakage into the system. Moreover dendrigraft can be easily regenerated with 0.1M NaCl to work as a reusable resin for water remediation.

Cross-linked dendrigraft (200-60)-TBAC showed non-specific bindings to nitrate, sulfate and perchlorate anions. Binding capacity of 3.32 meq/g was observed for perchlorate. Dendrigraft-anion complex was sufficiently strong to prevent leaching in the system and can be regenerated with low strength NaCl (0.1M). This total capacity is comparatively low for dendrigrafts; this is likely due to the large butyl groups blocking access of perchlorate to more binding sites inside the internal pores of the resin.

8. Biofouling

Biofouling was determined by measuring the thickness of a crosslinked G1(200-60)-TMAC film which has been incubated with a solution of bovine serum albumin proteins for various amounts of time. The silicon wafer was etched with 0.5M HF to increase adhesion of the dendrigraft film. The layer was washed with 10 mL of PBS buffer three times to remove unspecifically-bound BSA. It was found that there was no specific binding of bovine serum albumin on dendrigraft (200-60)-TMAC after 24 hours, which suggests that microbial growth should not occur.

Conclusions and Implications for Future Research and Implementation

The highlights of this technology evaluation for SERDP funded research focused on perchlorate removal in ground water remediation applications. Specific findings include:

1. A wide variety of candidate dendritic polymers were synthesized and all showed perchlorate binding. This is the first time these types of dendritic polymers have been made and tested for this application.
2. A few candidates with the best perchlorate binding capacity were tested for perchlorate, nitrate, and sulfate binding.
3. A cross-linking system to create resins that could be used in existing fixed-bed resin systems was created.
4. Binding capacities over 300 meq/g for perchlorate (in a UF membrane set-up), which is over 300x the capacity relative to many commercial resins (around 1 meq/g), were observed.
5. Cross-linked dendrigraft demonstrated 3.32 meq/g binding capacity in a fixed bed set-up for perchlorate.
6. Dendrigrafts experiments showed selective perchlorate binding over nitrate and sulfate.

7. Scale-up optimization was done to reduce the manufacturing costs of dendrigrafts. This included making 10g of dendrigraft without the UF purification procedure.
8. Operational performance and regeneration performance were investigated. It was found that the crosslinked dendrigraft backbone withstood 5 psi of pressure, and the pressure did not rise over 24 hour.
9. Dendrigrafts are regenerable with low strength (0.1M) NaCl.
10. Dendrigraft did not show significant leakage of perchlorate, sulfate, or nitrate anions when the exhausted, used column was washed with 72 bed volumes of water.

Dendrigrafts were tested with high perchlorate concentrations to ensure that even in the presence of high nitrate and sulfate concentrations the capacity for perchlorate is still high. With large capacities the resin is expected to be effective for the removal of low or high concentrations of perchlorate. The effective binding of low concentrations of perchlorate will be confirmed in future work.

Total capacity for insoluble crosslinked dendrigraft G1(200-60)-TBAC was found to be 3.32 meq/g, but that value will likely increase when tested with smaller volumes of resin and larger amounts of perchlorate. Also, the crosslinking density of this resin was high, which may have reduced the amount of perchlorate binding. Additionally, the TMAC resin will be measured as well. Even though literature shows that TBAC is more selective than the smaller TMAC, binding sites are not only on the surface but also throughout the resin volume. It is likely that the large binding site, especially in combination with the high amount of crosslinking, restricts access too much to the binding sites. Data shows that specificity is actually higher for TMAC resins than TBAC resins, which suggests that the restricted space of the internal pores increases selectivity for perchlorate without the need of the TBAC binding site.

It was also found that brine with a concentration of only 0.1M NaCl is needed for regeneration of this resin. The actual concentration needed might be significantly lower, and future regeneration experiments with decreasing brine concentrations will determine the lowest feasible concentration.

Future Research and Implementation includes:

1) Optimization of the operational parameters for G1(200-60)-TMAC dendrigraft for the perchlorate removal from the source water.
2) Pilot plant study to simulate operational conditions.
   Source water such as from the DOD testing facility such as the National Environment Technology test site at Dover Air Force Base will be used with the crosslinked G1(200-60)-TMAC dendrigraft. Flow rates up to 12 gpm/cu.ft. will be attempted. A breakthrough curve will be determined with continuous flow; a rest period will be considered depending on the results of the continuous use test. This resin will be tested as a regenerable resin and low concentration brine will be used for regeneration.
3) Procedures to lower the manufacturing cost further.

4) Evaluating the compatibility of crosslinked dendrigraft with other existing technologies so that dendrigraft technology can be used with the existing technologies to increase their performance and hence avoiding the cost associated with the replacement.
5) Verifying that dendrigraft technology can remove toxic or precious materials from the source fluid.

Literature Cited

1. Urbansky E.T., Perchlorate as an Environmental Contaminant. *Environmental Science and Pollution Research* **2002,** *9* (3), 197-192.
2. Logan B.E., Assessing the Outlook for Perchlorate Remediation. *Environmental Science and Technology* **2001,** *35* (23), 482A-487A.
3. Urbansky Edward Todd, Perchlorate as an Environmental Contaminant. *Environmental Science and Pollution Research* **2002,** *9* (3), 197-192.
4. Logan Bruce E., Assessing the Outlook for Perchlorate Remediation. *Environmental Science and Technology* **2001,** *35* (23), 482A-487A.
5. Matos Christina T.; Velizarov Svetlozar; Crespo Joao G.; Reis Maria A.M., Simultaneous Removal of Perchlorate and Nitrate from Drinking Water Using Ion Exchange Membrane Bioreactor Concept. *Water Research* **2006,** *40*, 231-240.
6. Matos Christina T.; Fortunato Raquel; Velizarov Svetlozar; Reis Maria A.M.; Crespo Joao G., Removal of Mono-Valent Oxyanions from Water in an Ion Exchange Membrane Bioreactor: Influence of Membrane Permselectivity. *Water Research* **2008,** *42*, 1785-1795.
7. Brown Gibert M.; Bonnesen Peter V.; Moyer Bruce A.; Gu Baohua; Alexandratos Spiro D.; Patel Vijay; Ober Robert, The Design of Selective Resins for the Removal of Pertechnetate and Perchlorate from Wastewater. In *Perchlorate in the Environment*, Urbansky Edward Todd, Ed. Kluwer Academic/Plenum Publishers: New York, 2000; pp 155-164.
8. Gu Baohua; Brown Gibert M.; Chiang Chen-Chou, Treatment of Perchlorate-Conaminated Groundwater Using Highly Selective, Regenerable Ion-Exchange Technologies. *Environmental Science and Technology* **2007,** *41*, 6277-6282.
9. Batista J.R.; McGarvey F.X.; Vieira A.R., The Removal of Perchlorate from Waters Using Ion-Exchange Resins. In *Perchlorate in the Environment*, Urbansky E.R., Ed. Kluwer Academic: New York, 2000; pp 135-145.
10. Tripp Anthony R.; Clifford Dennis A., Ion Exchange for the Remediation of Perchlorate-Contaminated Drinking Water. *Journal of the American Water Works Association* **2006,** *98* (4), 105-114.
11. Xiong Zhong; Dimick Paul; Zhao Dongye; Kney Arthur; Tavakolu Javad, Removal of perchlorate from Contaminated Water Using a Regenrable Polymeric Ligand Exchanger. *Separation Science and Technology* **2006,** *41*, 2555-2574.
12. Diallo Mamadou S.; Kwesi., F.; Johnson James H. Jr.; Goddard William A., Dendritic Anion Hosts: Perchlorate Uptake by G5-NH2 Poly(propylenimine) Dendrimer in Water and Model Electrolyte Solutions. *Environmental Science and Technology* **2007,** *41*, 6521-6527.
13. Xiong Zhong; Zhao Dongye; Harper Willie F. Jr., Sorption and Desorption of Perchlorate with Various Classes of Ion Exchangers: A Comparative Study. *Industrial and Engineering Chemical Research* **2007,** *46*, 9213-9222.
14. Egorov V.V.; Rakhman'ko E.M.; Pomelenok E.V.; Okaev E.B., Effect of the Steric Acessibility of the Exchange Site in Long-Chain Quaternary Ammonium Salts on the Anion Exchange Extraction of Divalent Ions. *Russion Journal of Physical Chemistry* **2006,** *80* (6), 969-973.

15. Brown G.M.; Bonnesen P.V.; Moyer B.A.; Gu B.; Alexandratos S.D.; Patel V.; Ober R., The Design of Selective Resins for the Removal of Pertechnetate and Perchlorate from Groundwater. In *Perchlorate in the Envieonment*, Urbansky E.T., Ed. Kluwer Academic: New York, 2000; pp 155-164.
16. Kesting Robert E., *Synthetic Polymeric Membranes*. McGraw-Hill: New York, NY, 1971.
17. Eisenbarth E., Biomaterials for Tissue Engineering. *Advanced Engineering Materials* **2007,** *9* (12), 1051-1060.
18. Jeon S. I.; Lee J. H.; Andrade J. D.; De Gennes P. G., Protein-Surface Interactions in the Presence of Polyethylene Oxide. I. Simplified Theory. *Journal of Colloid and Interface Science* **1991,** *142* (1), 149-58.
19. Li W.-H.; Stover H.D.H., Monodisperse poly(chloromethylstyrene-co-divinylbenzene) microspheres by precipitation polymerization. *Journal of Polymer Science: Part A: Polymer Chemistry* **1999,** *37*, 2295-2303.
20. Kolb H.C.; Finn M.G.; Sharpless K.B., Click Chemistry: Diverse Chemical Function from a few Good Reactions. *Angewandte Chemie, International Edition in English* **2001,** *40*, 2004-2021.

Appendices

A. Supporting Data:
No additional data to report.

B. List of Scientific/Technical Publications:
- Veera Reddy Pulgam, Hosam G. Abdelhady, Syed A. Ashraf, Abhay Chauhan, Ryan Hayes, Dillip Mohanty, Xuezheng Song, Sonke Svenson, Douglas R. Swanson, Jie Wang, Michael A. Zhuravel, Anja Mueller* "A Dendrigraft Resin for Perchlorate Removal", Water Science and Technology, submitted.
- Anja Mueller, Sonke Svenson, Dillip Mohanty, Hosam G. Abdelhady, Syed A. Ashraf, Veronica G. Frawley, Xuezheng Song, Douglas R. Swanson, Jie Wang, Michael A. Zhuravel "Perchlorate Remediation Using New Nanoscale Dendritic Polymer Technology", Water & Industry 2009, Palmerston North, December 2009, Proceedings, accepted.
- Abhay Chauhan*, Anja Mueller, Veera Reddy Pulgam, Douglas R. Swanson, Jie Wang, Ryan Hayes, Hosam G. Abdelhady, Sonke Svenson, Dillip Mohanty, Syed A. Ashraf, Veronica G. Frawley, Xuezheng Song, Michael A. Zhuravel "A Dendrigraft Resin for Perchlorate Removal ", Environmental Technology Technical Symposium & Workshop 2009, Washington D.C., December 2009, Abstract accepted.